Journal of Agricultural and Crop Research
Vol. 9(11), pp. 264-267, November 2021
doi: 10.33495/jacr_v9i11.21.166
ISSN: 2384-731X
Research Paper

Diversity and frequency of fungal genera from sorghum lines inoculated with *Alternaria alternata* alone and in combination with *Curvularia lunata* and *Fusarium thapsinum* in a field infected with anthracnose

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Accepted 29th October, 2021.

Abstract. Sorghum grain mold is a disease associated with number of fungal genera. In this study, mycoflora analysis of seeds obtained from 91 accessions, including those from the sorghum association panel was conducted. Accessions were inoculated with *A. alternata* alone, mixture of *A. alternata*, *F. thapsinum*, and *C. lunata*, and water-sprayed control during two growing seasons. *Alternaria alternata* alone treatment showed *Colletotrichum sublineola* was the most recovered fungal species followed by *A*. *alternata*, and *Fusarium* spp. Seeds from panicles inoculated with a mixture of *A*. *alternata*, *F*. *thapsinum*, and *C*. *lunata* revealed *F*. *thapsinum*, followed by *C. lunata*, *C. sublineola* and *A*. *alternata* as the most frequently isolated species. In the control treatment, *A*. *alternata* and *C. sublineola* were the most frequently recovered fungal species. *Fusarium proliferatum*, *Aspergillus flavus*, *A*. *niger*, *F*. *chlamydposporum*, *F*. *verticillioides*, *Rhizopus* spp., and *Penicillium* spp. were also recovered but in low percentages. Due to the large number of fungi associated with the disease, management can be challenging. Studies like this allows for the identification of other grain mold fungi infecting or contaminating sorghum grain in a field. Thus, using those fungi species in a particular region, either individually or in combination to screen germplasm for resistance or tolerance to this disease complex is recommended.

Keywords: Mycoflora, Sorghum, Grain Mold, Fungal Species, *Alternaria alternata*, *Fusarium thapsinum*, *Curvularia lunata*.

INTRODUCTION

Globally, sorghum is the fifth most important cereal in the drier tropics, the crop provides the calorie intake needs for millions of people (Frederiksen and Odvody, 2000). Sorghum production and the ability of farmers to generate profit and sustenance from the crop are hampered by both abiotic and biotic stresses. One of those biotic stresses is grain mold, a complex fungal disease considered to be the most important sorghum disease worldwide. Sorghum grain mold is associated with number of fungi genera, including *Fusarium thapsinum* Klittick, Leslie, Nelson, Manasas (Klittich *et al*., 1997); *Fusarium semitectum* Berk. & Ravenel; *Curvularia lunata* (Wakk.) Boedijn; *Colletotrichum sublineola* Henn ex Sacc & Trotter; *Alternaria alternata* (Fr.: Fr.) Keissl.; and *Phoma sorghina* (Sacc.) Boerema, Dorenbosch, & Van Kesteren (Esele *et al*., 1995; Singh and Bandyopadhyay, 2000). These fungal genera, particularly *Fusarium* species have the capacity to produce mycotoxins either in the field or when the grain is stored under poor conditions (Sashidha *et al*., 1992; Leslie *et al*., 2005; Isakeit *et al*., 2008; Little *et al*., 2012).

The disease is most severe in areas where frequent rainy conditions occur later in the growing season and where mature grains are not harvested on time (Singh and Bandyopadhyay, 2000; Thakur *et al.*, 2007). Yield losses in grain yield on highly susceptible sorghum lines can reach 100 % (Navi *et al.*, 2005). Another factor that makes

management of grain mold challenging is that fungi infecting or contaminating sorghum grain vary across years and locations (Prom *et al.*, 2015; Nagaraja *et al.*, 2016, Taylor and Ngaujah, 2016, Osman *et al.*, 2017). Thus, this study was conducted in 2014 and 2015 to determine the frequency of isolation of different fungi on sorghum grain inoculated with *Alternaria alternata* alone, in combination with C*. lunata* and *F. thapsinum,* and water-sprayed control in an anthracnose infected field.

MATERIALS AND METHOD

The 91 accessions, including part of the sorghum association panel (SAP) compiled by Casa *et al*. (2008) and maintained by the USDA-ARS, Plant Genetic Resources Conservation Unit, Griffin, Georgia, were used in this study. The accessions were inoculated with *A. alternata* alone, a mixture of *A. alternata, F. thapsinum, and C. lunata*, and a control sprayed with sterile water. Accessions were evaluated in the 2014-2015 growing seasons in a randomized complete block design and replicated three times at the AgriLife Research Farm, Burleson County, TX. Standard field preparations such as fertilizer application and other agronomic managements were followed.

Treatment: Inoculum preparation and inoculation method were as previously described by Prom *et al.* (2003). Nine panicles at 50 % bloom were arbitrarily selected and tagged from each row then inoculated using a hand sprayer, three panicles per treatment [three treatments: a) *A. alternata* alone; 2) mixture of *A. alternata*, *F. thapsinum*, and *C. lunata*; and 3) untreated control]. The untreated control panicles were sprayed with sterile distilled water. Inoculated and the water-sprayed control panicles were covered with paper bags for 24 h. To enhance infection and disease development, treated and water-sprayed control panicles were misted twice a day (once in the morning and afternoon) for 7 consecutive days. At maturity, treated and control panicles were harvested and hand threshed.

Mycoflora analysis: The protocol for determining sorghum seed mycoflora had been previously described by Prom *et al.* (2003) and Prom (2004). Briefly, 227 samples were analyzed across the treatments. Fifty surface sterilized seeds per sample were plated on Petri dishes containing half-strength potato dextrose agar and incubated at 25±2°C for 7 days. Microscopic identification of fungal species was based on the conidia, conidiophores, colony morphology, and color, according to descriptions provided by Booth (1971), Nelson *et al*. (1983), and Barnett and Hunter (1998).

RESULTS AND DISCUSSION

Fungal communities isolated from seeds obtained from sorghum accessions, including Sorghum Association Panel (SAP) are noted in Table 1. Across years, seeds obtained from *A*. *alternata* alone treatment showed *Colletotrichum sublineola* (25%) was the most recovered fungal species, followed by *A*. *alternata* (23%), *Fusarium* spp. (12.5%), *F*. *semitectum* (9.5%), *C*. *lunata* (7.0%), *F*. *thapsinum* (5.0%), and *Bipolaris* spp. (4%) (Table 1). *Fusarium proliferatum*, *Aspergillus flavus*, *A*. *niger*, *F*. *chlamydposporum*, *F*. *verticillioides*, *Rhizopus* spp., and *Penicillium* spp. were recovered in low percentages. Seeds inoculated with a mixture of *A*. *alternata*, *F*. *thapsinum*, and *C*. *lunata* revealed *F*. *thapsinum* (34.8%), *C. lunata* (16.7%), *C. sublineola* (14%), and *A*. *alternata* (12%) as the most frequently isolated species. Other genera and/or species such as *Fusarium* spp., *F*. *semitectum*, *F*. *proliferatum*, *Bipolaris* spp., were also isolated. In the control treatment, *A*. *alternata* (25.7%) and *C. sublineola* (18.2%) were the most frequently recovered fungal species, followed by *Fusarium* spp., *F*. *semitectum*, *C. lunata. Bipolaris* spp*., F*. *proliferatum* and *F*. *thapsinum*. The high recovery of *C. sublineola*, causal agent of sorghum anthracnose was expected because the plants in the field were inoculated with this pathogen at the 8-leaf stage of development and spores from infected leaves splashed onto the panicles during and later in the season. In the untreated control, *Alternaria alternata* (25.7%) was the most frequently recovered species, followed by *C. sublineola*. The dominance of *Alternaria alternata* on naturally infected sorghum seeds had been reported in several mycoflora studies. Across several Counties in South Texas, Prom *et al*. (2015) noted that *Alternaria* spp. was the dominant fungal species isolated from naturally infected sorghum seeds. Also, *Alternaria alternata* was the most frequently recovered fungal species on sorghum seeds collected from different locations in Turkey (Turgay and Ünal, 2009), while *Alternaria* spp. was isolated from all sorghum seed samples collected from 14 locations in Eritrea, Northeast Africa (Naqvi *et al*., 2013). In Burleson County, Texas, *Alternaria* spp. and *F*. *semitectum* were the most frequently recovered fungi species on naturally infected sorghum seeds (Prom, 2004). In addition, 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 (Prom *et al*., 2021). Similar to this work, other fungal species, including *C. lunata*, *Bipolaris* sp., *C. sublineola*, *F*. *verticillioides*, *Penicillium* sp., *Aspergillus flavus, F. thapsinum*, *F. oxysporum*, *F*. *sporotrichioides*, *F*. *graminearum*, *F*. *proliferatum*, and *A. niger* were also isolated from sorghum seeds collected from farmers' fields in Texas in various frequencies by Prom *et al*. (2021). Unlike previous mycoflora studies in Texas, sorghum seed samples collected from Florida and Georgia, found *F. incarnatum, F. acuminatum, F. equiseti, & F. semitectum* Complex as the most frequently recovered fungal species (Prom *et al*., 2021). Erpelding and Prom (2006) reported that *F*.

Table 1. Mean % frequency of fungal isolates recovered across sorghum lines and treatments during the 2014-2015 evaluation periods[1]

	Treatment		
Fungal isolate	***Alternaria alternata* alone**	**Mixture (*A. alternata*+*F. thapsinum*+*C. lunata*)**	**Control**
Alternaria alternata	23	12	25.7
Aspergillus niger	0.23	0.08	0.95
Aspergillus flavus	1.0	0.8	0.49
Bacteria*	3.6	1.0	2.13
Bipolaris spp.	4.0	2.0	3.9
Colletotrichum sublineola	25	14	18.2
Clean**	2.2	1.0	2.9
Curvularia lunata	7.0	16.7	7.3
Epicoccum sorghinum	0.3	0.22	0.59
Fusarium proliferatum	2.0	3.0	3.2
Fusarium semitectum	9.5	4.9	8.8
Fusarium thapsinum	5.0	34.8	2.9
Fusarium chlamydosporum	0.1	0	0.1
Fusarium verticillioides	0.4	0.5	0.8
Fusarium spp.	12.5	7.0	12.07
Rhizopus spp.	0.25	0.2	1.1
Unknown***	3.82	1.8	8.9
Penicillium spp.	0.1	0	0

[1]Sorghum accessions were planted in 2014-2015 growing seasons at the AgriLife Research Farm, Burleson County, TX. Seed mycoflora analysis across accessions showing the percent recovery of the various fungal species among the three treatments [*Alternaria alternata* alone, mixture (*A. alternata* + *Fusarium thapsinum* + *Curvularia lunata*), and control (panicles sprayed with sterile water)].

semitectum was the dominant fungal species infecting or contaminating sorghum seed samples collected from Isabela, Puerto Rico, during the 2002 and 2003 growing seasons. The number of fungal genera associated with sorghum grain mold disease complex and their variability on mature sorghum grain make management challenging. One strategy for control may be to identify the most common fungi infecting sorghum in a region and using them either individually or combined to screen germplasm for resistance or tolerance to this disease complex.

ACKNOWLEDGEMENT

This research (CRIS # 3091-22000-034-00D) was supported by the U. S. Department of Agricultural Research Service.
USDA is an equal opportunity provider and employer

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