Development of a reliable inoculation technique to assess resistance in pearl millet to *Fusarium* grain mold

SK Nutsugah[1] and **JP Wilson**[2]*

1. Savanna Agricultural Research Institute, PO Box 52, Nyankpala, Tamale, Ghana
2. USDA-ARS Crop Genetics and Breeding Research Unit, University of Georgia, Tifton, Georgia, USA

*Corresponding author: jeff.wilson@ars.usda.gov

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Abstract

Pearl millet is an alternative grain for the drought-prone southeast region of the United States. High humidity in this region can frequently promote the development of diverse fungi associated with grain mold complex. This study was conducted to develop a reliable method for grain mold inoculations, and to assess the grain mold resistance of pearl millet inbreds. A mixture of *Fusarium semitectum*, *F. chlamydosporum*, and *F. verticillioides* was used to inoculate seven pearl millet inbreds, Tift 454, Tift 99B, 106B, 206B, 406B, 506B and 606B in the greenhouse. Mold growth was visible at 3 days, and increased during the 7-day incubation period. Mold severity ratings did not differ when evaluated at 2 or 4 weeks after inoculation. More precise control over environmental conditions during the incubation period should be explored for more consistent results across inoculation dates. Inbreds differed in their susceptibility to grain molds. Tift 99B, Tift 454 and 606B were most susceptible inbreds, whereas 506B and 106B were the most resistant. These experiments identified a combination of inoculation treatment and incubation technique that was sufficient for the reliable development of grain mold in pearl millet for breeding, inheritance and quality studies.

Introduction

Pearl millet (*Pennisetum glaucum*) is an alternative grain crop for the drought-prone southeast region of the United States. The grain is a high-quality addition to poultry rations (Davis et al. 2003) and has a ready market in bobwhite quail (*Colinus virgianus*) rations for the recreational wildlife industry. Grain and seed molds commonly occur when a pearl millet crop matures during times of high humidity or excessive rainfall. *Fusarium semitectum* and *F. chlamydosporum* are common grain mold fungi in Georgia, USA (Wilson et al. 2006). Infected grain may possess poor nutritional qualities or may harbor fungi that could produce potentially harmful mycotoxins such as trichothecenes and zearalenone (Wilson et al. 1993).

Field and laboratory screening techniques have been used to assess a diverse global pearl millet germplasm collection for grain mold resistance (Navi et al. 2006) but controlled inoculation techniques have not been developed for pearl millet. Because environmental conditions play an important role in the prevalence of grain mold fungi (Wilson et al. 1993, 1995), development of controlled inoculation techniques would presumably allow more precise identification of resistance. The present study was conducted to develop a reliable method for grain mold inoculations, and to assess the grain mold resistance of pearl millet inbreds inoculated with a mixture of *F. semitectum*, *F. chlamydosporum* and *F. verticillioides*.

Materials and methods

Experiments were conducted at the University of Georgia Tifton, Georgia, USA. Seven pearl millet inbreds, Tift 454, Tift 99B, 106B, 206B, 406B, 506B and 606B, were evaluated in a series of four inoculations in the greenhouse. Fifteen pots of each inbred were planted at 14-day intervals for four plantings. Stands were thinned to one plant per pot. Plants were prepared for inoculation shortly after anthesis during early grain fill. Anthers were lightly brushed off using a test-tube brush to expose the developing grains. Fungal inoculation treatments used in this study were a mixture of *F. semitectum*, *F. chlamydosporum* and *F. verticillioides*, or a water-sprayed control. The number of replications in each inoculation was determined by the availability of an adequate number of plants at the correct growth stage on a given day when inoculations were performed. Ten panicles per inbred were used for the fungal inoculation treatment and two panicles for the water control treatment in the first three inoculations. In the fourth inoculation, five panicles per inbred were used for the fungal treatment and two panicles for the control treatment. Within each inoculation date, inoculated inbreds were arranged in a completely

randomized design and each panicle was considered a replication.

Cultures of *F. semitectum*, *F. chlamydosporum* and *F. verticillioides* were isolated from plated pearl millet grain and were increased separately on potato-dextrose agar incubated at 25°C for 10–14 days under continuous fluorescent light. Fungal spores were harvested by flooding the plates with sterile purified water and gently scraping the agar surface with rubber spatula to dislodge the conidia. Spores and mycelial fragments in the resulting suspensions were counted with a hemacytometer and diluted with sterile purified water to make 1.5 L of inoculum suspension containing approximately 9×10^6 spores and mycelial fragments/ml. Suspensions of the three fungi were made separately. For the mixture, equal volumes (1:1:1 v/v/v) of each of the three fungal suspensions were combined in a flask. Thus, the mixed fungal suspension had approximately 3×10^6 spores and mycelial fragments of each fungus/ml. Panicles were inoculated on 2 March 2007 for the first inoculation, 6 March 2007 for the second, 7 March 2007 for the third and 8 March 2007 for the fourth. Inoculum was sprayed onto panicles with a hand-held spray bottle which was thoroughly agitated during the procedure. The panicles were sprayed to run-off. The non-inoculated panicles were similarly sprayed with water. Immediately after inoculation, panicles were covered with pre-wetted plastic bags which remained in place for a 7-day incubation period.

Panicles were evaluated for mold severity at 2 and 4 weeks after inoculation. Ratings were assigned on a scale of 0–5 where 0 = no mold visible, 1 = scant superficial mold growth and up to 10% of grain and panicle surface covered by mold, 2 = moderate mold growth and 11–25% of grain and panicle surface molded, 3 = considerable mold growth and 26–50% of grain surface molded, 4 = extensive mold growth and 51–75% of grain and panicle surface molded, and 5 = extensive mold growth and more than 76% of grain and panicle surface molded. In the analysis of variance of mold ratings, sums of squares were partitioned into inoculation date, replication, evaluation date, inbred, and two- and three-way interactions among inoculation date, evaluation date and inbred. Means were separated using Fisher's LSD at $P \leq 0.05$.

Results and discussion

The inoculation and prolonged incubation treatment successfully resulted in grain mold development. Mold growth was visible at three days, and increased during the incubation period. The average score of the inoculated plants was 3.0. A limited amount of mold developed in the water-treated control, probably due to infection from background inoculum levels in the greenhouse coupled with the increased relative humidity from the plastic bag incubation technique. Grain mold ratings averaged 0.7 for the control plants, which differed ($P < 0.001$) from mold levels of the inoculated plants. Ratings were not affected by evaluation date, and averaged 3.0 when assessed at either 2 or 4 weeks after inoculation. Evaluation date × inbred and inoculation × evaluation date × inbred interactions were not significant ($P > 0.05$).

Table 1. Grain mold development on pearl millet inbreds inoculated in the greenhouse with a mixture of *Fusarium semitectum*, *F. chlamydosporum* and *F. verticillioides*.

Inbred	Grain mold rating[1]
Tift 99B	3.8 a
Tift 454	3.6 a
606B	3.6 a
206B	2.8 b
406B	2.6 bc
106B	2.4 cd
506B	2.3 d
LSD ($P \leq 0.05$)	0.3

1. Data were pooled for ratings taken at 2 and 4 weeks after inoculation. A score of 0 = no mold visible on grain surface; 1 = up to 10% of grain surface covered by mold; 2 = 11–25% of grain surface molded; 3 = 26–50% of grain surface molded; 4 = 51–75% of grain surface molded; and 5 = >76% of grain surface molded. Values followed by the same letter are not significant.

Mean grain mold ratings differed for each inoculation date ($P < 0.001$). Mean ratings across inoculation dates ranged from 2.6 for the third inoculation date to 3.5 for the second. This level of difference is likely to cause some difficulties in experiments requiring single plant inoculations over multiple dates. More precise control over environmental conditions during the incubation period should be explored to produce more consistent results across inoculation dates.

Inbreds differed in their susceptibility to grain molds. Tift 99B, Tift 454 and 606B were most susceptible inbreds, whereas 506B and 106B were the most resistant (Table 1). This information will be useful in decisions concerning germplasm releases, and for further breeding efforts for grain mold resistance. These experiments identified a combination of inoculation treatment and incubation technique that was sufficient for the reliable development of grain mold in pearl millet. This technique will be useful in germplasm screening and breeding applications, and in inheritance studies, particularly before using more intensive analyses such as that proposed by Chintapalli et al. (2006). In the present study, *Fusarium* spp common on pearl millet in southeastern United States were assessed because of their potential to produce mycotoxins that would affect the quality of grain. Many other fungi can cause grain molds in other regions of the world (summarized by Wilson

2000). This incubation technique should be applicable to screening for resistance to most grain mold fungi of pearl millet.

Little is known about the mechanisms for grain mold resistance in pearl millet (Chandrashekar and Satyanarayana 2006). The in vitro antifungal activity was assessed for a cysteine protease inhibitor extracted from pearl millet seeds (Joshi et al. 1998), but its role in differential grain mold resistance among pearl millet genotypes is not presently known. The present study is the first report of resistance to *Fusarium* grain molds resulting from controlled inoculations. These experiments identified a combination of inoculation treatment and incubation technique that can reliably produce grain mold in pearl millet for resistance, breeding, inheritance and quality studies.

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References

Chandrashekar A and **Satyanarayana KV.** 2006. Disease and pest resistance in grains of sorghum and millets. Journal of Cereal Science 44:287–304.

Chintapalli R, Wilson JP and **Little CR.** 2006. Using fungal isolation rates from pearl millet caryopses to assess grain mold and weathering resistance. International Sorghum and Millets Newsletter 47:146–148.

Davis AJ, Dale NM and **Ferreira FJ.** 2003. Pearl millet as an alternative feed ingredient in broiler diets. Journal of Applied Poultry Research 12:137–144.

Joshi BN, Sainani MN, Bastawade KB, Gupta VS, and **Ranjekar PK.** 1998. Cysteine protease inhibitor from pearl millet: A new class of antifungal protein. Biochemical and Biophysical Research Communications 246:382–387.

Navi SS, Tonapi VA, Varanavasiappan S and **Ravinderreddy Ch.** 2006. Host plant resistance to grain mould in germplasm accessions of pearl millet (*Pennisetum glaucum* [L.] R. Br.). Archives of Phytopathology and Plant Protection 39:465–477.

Wilson JP. 2000. Pearl millet diseases: A compilation of information on the known pathogens of pearl millet. USDA-ARS Agricultural Handbook no. 716. USA: USDA.

Wilson JP, Casper HH and **Wilson DM.** 1995. Effect of delayed harvest on contamination of pearl millet grain with mycotoxin-producing fungi and mycotoxins. Mycopathologia 132:27–30.

Wilson JP, Hanna WW, Wilson DM, Beaver RW and **Casper HH.** 1993. Fungal and mycotoxin contamination of pearl millet grain in response to environmental conditions in Georgia. Plant Disease 77:121–124.

Wilson JP, Jurjevic Z, Hanna WW, Wilson DM, Potter TL and **Coy AE.** 2006. Host-specific variation in infection by toxigenic fungi and contamination by mycotoxins in pearl millet and corn. Mycopathologia 161:101–107.