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Short communication

Evaluation of maize inbred lines for resistance to pre-harvest aflatoxin and fumonisin contamination in the field

Baozhu Guo[a,*], *Xiangyun Ji*[b,c], *Xinzhi Ni*[d], *Jake C. Fountain*[b], *Hong Li*[e], *Hamed K. Abbas*[f], *Robert D. Lee*[g], *Brian T. Scully*[h]

[a]*United States Department of Agriculture-Agricultural Research Service (USDA-ARS), Crop Protection and Management Research Unit, Tifton, GA, United States*
[b]*The University of Georgia, Department of Plant Pathology, Tifton, GA, United States*
[c]*Ecological Environment Protection Research Institute, Shanghai Academy of Agricultural Sciences, Shanghai, China*
[d]*USDA-ARS, Crop Genetics and Breeding Research Unit, Tifton, GA, United States*
[e]*Shanxi Academy of Agricultural Sciences, Millet Research Institute, Changzhi, China*
[f]*USDA-ARS, Biological Control of Pests Research Unit, Stoneville, MS, United States*
[g]*The University of Georgia, Department of Crop and Soil Sciences, Tifton, GA, United States*
[h]*USDA-ARS, U.S. Horticultural Research Laboratory, Fort Pierce, FL, United States*

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ABSTRACT

Two important mycotoxins, aflatoxin and fumonisin, are among the most potent naturally occurring carcinogens, contaminating maize (*Zea mays*) and affecting crop yield and quality. Resistance of maize to pre-harvest mycotoxin contamination, specifically aflatoxin produced by *Aspergillus flavus* and fumonisin produced by *Fusarium verticillioides*, is a goal in breeding programs that screen for these important traits with the aim of developing resistant commercial hybrids. We conducted two years of field evaluations on 87 inbred lines originating primarily in China and Mexico and not previously screened for resistance. The objectives of our study were to identify resistant germplasm for breeding purposes and to examine possible relationships between resistances to the two mycotoxins. Aflatoxin and fumonisin were present in samples harvested from all lines in both years. Concentrations of total aflatoxin ranged from 52.00 ± 20.00 to 1524.00 ± 396.00 μg kg^{-1}, while those of fumonisin ranged from 0.60 ± 0.06 to 124.00 ± 19.50 mg kg^{-1}. The inbred lines TUN15, TUN61, TUN37, CY2, and TUN49 showed the lowest aflatoxin accumulation and CN1, GT601, TUN09, TUN61, and MP717 the lowest fumonisin accumulation. TUN61 showed the lowest accumulation of both mycotoxins. This study confirmed previous observations that high levels of aflatoxin can coexist with fumonisin, with 55 maize lines showing a positive correlation coefficient between the concentrations of aflatoxin and fumonisin and 32 lines showing a negative correlation coefficient. These selected lines,

* *Corresponding author.*
E-mail address: baozhu.guo@ars.usda.gov (B. Guo).
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particularly TUN61, may provide sources of resistance to mycotoxin contamination in breeding programs. However, the mechanism of resistance in this germplasm remains to be identified. Future research should also address factors that influence the fungus–plant interaction, such as herbivory and environmental stress.

1. Introduction

Aspergillus flavus (Link ex Fr.) and *Fusarium verticilliodes* (Sacc.) Nirenberg [syn.: *F. moniliforme*] are the two predominant ear-rotting pathogens of maize (*Zea mays* L.) in the southern U.S. Damage caused by ear-feeding pests can provide an entrance for fungal infection leading to subsequent mycotoxin contamination (such as by aflatoxin and fumonisin) of grain. Mycotoxin contamination results in severe yield losses, reduces crop quality [1], and poses a significant threat to human and animal food safety [2–5]. Aflatoxins are powerful hepatotoxins, teratogens, mutagens, and carcinogens [3,4,6], and fumonisins have been reported to induce several diseases in animals, notably leukoencephalomalacia in horses, pulmonary edema in swine, and liver cancer in human [7–11]. The U.S. Food and Drug Administration (FDA) regulates aflatoxin B_1, the most common form of aflatoxin found in maize, at less than 20 $\mu g\ kg^{-1}$ for human consumption, whereas total fumonisin levels in human food and animal feed are regulated at less than 2 $mg\ kg^{-1}$ [12,13].

Although both mycotoxins can be found in the same maize ear, the relationship between aflatoxin and fumonisin contamination is not well understood. Marin et al. [14] found that the growth of *Aspergillus* spp. was slowed in the presence of *Fusarium* spp., showing a competitive relationship and a negative correlation between the growth of *A. flavus* and *F. verticillioides* [14]. This observation was supported by Zummo and Scott [15] who found lower levels of aflatoxin in ears inoculated with both fungi than in ears inoculated with *A. flavus* alone. In contrast, Abbas et al. [16] found that aflatoxin and fumonisin levels were positively correlated across test environments in hybrids naturally infected with both *Fusarium* spp. and *A. flavus*. These results suggest that both fungi can thrive on similar resources if host plants are highly susceptible.

Breeding techniques employed for maize have developed and advanced over time and include conventional breeding, mutation breeding, and molecular-assisted breeding (including transgenics and molecular markers). Conventional breeding has made a significant contribution to increased maize production through the development of hybrids with resistance to abiotic stresses such as drought, heat, and cold, and with partial resistance to aflatoxin and fumonisin [17,18]. Screening of maize lines for aflatoxin resistance has been performed using several methods of inoculation, such as side-needle inoculation or pinbars [19]. Laboratory kernel screening assay (KSA) has also been used for rapid screening, though the results of KSA are not always consistent with observed resistance in the field [20]. Studies of fumonisin accumulation often rely on natural *Fusarium* spp. infection rather than on artificial inoculation [16].

Quantification techniques for aflatoxin and fumonisin have also advanced from thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) to more rapid, high-throughput methods such as enzyme-linked immunosorbent adsorption assay (ELISA) [21]. Identification of resistant germplasm for breeding purposes is still considered to be one of the best strategies available to lower aflatoxin and fumonisin accumulation in maize in the field [22–24].

Efforts to develop inbred lines resistant to aflatoxin contamination in grains have been ongoing since the mid-1970s. Henry et al. [25] conducted a two-year experiment to identify maize inbred lines resistant to both *A. flavus* and *F. verticillioides* and found that Mp717 showed low levels of aflatoxin and fumonisin [22,25]. Some sources of resistance to aflatoxin and fumonisin contamination have been identified by screening for aflatoxin and fumonisin contamination following inoculation with *A. flavus* and *F. verticillioides*, respectively [25,26]. However, few data are available for maize inbred lines with resistance to both *A. flavus* and *F. verticillioides*, and novel sources of resistance to contamination with either mycotoxin are limited in breeding programs. Accordingly, the objectives of this study were to screen the available inbred lines in our breeding program in Tifton, Georgia for resistance to aflatoxin and fumonisin accumulation, and to determine whether there was a correlation between aflatoxin and fumonisin resistance in the selected inbred lines. The evaluation and identification of maize lines with reduced aflatoxin and fumonisin contamination will assist in breeding for improvement of maize resistance to mycotoxin contamination.

2. Materials and methods

2.1. Maize inbred lines

Eighty-seven maize inbred lines were selected, largely Chinese lines (designated as TUN, CN, CM, and CY), and grown at the Belflower Research Farm, Tifton, Georgia, in 2007 and 2008. The resistant controls were Mp717 [22,25], Tex6 [26,28], GT601 to GT603 [29,30]. In both years, GTP27 was used as a susceptible control [30,31]. The field study was conducted in a randomized complete block design with at least six replications. Experiments were planted in a Tifton loamy sand (fine-loamy, siliceous, thermic Plinthic Kandiudult), and irrigation was performed as needed. Recommended crop management practices were applied in all field plots. Each plot consisted of two rows 3.0 m in length with a 1.0 m alley between plots, and was thinned to 20 plants per row.

2.2. Fungal inoculation

The *A. flavus* isolate NRRL3357 was cultured on V8 agar plates at 32 °C for 5–7 days prior to use in the experiment. Conidia were washed from the plates in 0.1% (v/v) Tween 20, and the concentration of conidia was determined with a hemocytometer