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Breeding Maize for Resistance to Mycotoxins at IITA

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Abstract

Ear-rot-causing fungi, including *Aspergillus* and *Fusarium* spp., are common in maize in West and Central Africa. These fungi contaminate maize with mycotoxins that pose serious potential health hazards to humans in these areas. A collaborative germplasm screening project was initiated between the International Institute of Tropical Agriculture (IITA) and the Southern Regional Research Center (SRRC) of the USDA's Agricultural Research Service in 1998 to develop maize germplasm with resistance to aflatoxin contamination. In a laboratory screen, some IITA inbred lines had potential levels of resistance to aflatoxin production as high as or higher than the best lines from the United States. These results prompted the initiation of a breeding project to combine resistance factors from the IITA lines with resistance factors from the US inbred lines. Several crosses and backcross populations were made from selected resistant or potentially resistant inbred lines from the US and IITA. Sixty-five S_5 lines were developed from the backcross populations and 144 S_5 lines were derived from the F_1 crosses. Kernels from these lines were screened in a laboratory assay. Significant differences in aflatoxin accumulation were detected amongst the lines within each group. Several S5 lines in which aflatoxin contamination was significantly less than in either parent were selected for resistance-confirmation tests. We found pairs of S_5 lines with 88-97% common genetic backgrounds that differed significantly in aflatoxin accumulation. These pairs of lines are being used for proteomic analyses to identify the proteins and the corresponding genes that limit aflatoxin accumulation. We also found significant differences in fumonisin accumulation amongst 58 elite maize inbred lines in which variation in aflatoxin accumulation was found. Both the new inbreds and the elite lines can be exploited as new genetic sources in breeding programs in which the objective is to develop maize cultivars/hybrids that accumulate lesser amounts of mycotoxins.

Introduction

Maize is a major staple food crop widely grown from the wet climate of the coast to the dry Sudanese savannas of West and Central Africa. The most common ear and kernel rot causing fungal species in maize in this area include *Aspergillus*, *Botryodiplodia*, *Diplodia* and *Fusarium* (Fajemisin *et al.*, 1985). Some of these fungi are widespread in different tropical maize growing

environments and can cause considerable economic losses by reducing grain quality in maize (Miller, 1996). When two of these fungal species, *Aspergillus flavus* and *Fusarium verticillioides*, infect maize plants, the developing kernels can be contaminated with aflatoxins and fumonisins, respectively, that render the harvested grain unfit for use as human food or animal feed.

Contamination with aflatoxins and fumonisins occur in warm, humid, tropical and subtropical maize-growing environments that are conducive to growth and development of the two fungi (Widstrom, 1996; Kpodo and Bankole, Chapter 9; Siame and Nawa; Chapter 10). Maize contaminated with mycotoxins is a serious problem in Sub-Saharan Africa because most of the maize grain in many countries is used for human consumption and the capacity to monitor the mycotoxin levels in the grain is limited (Widstrom, 1996). The widespread exposure to aflatoxin in Africa has been implicated in the increased incidence of acute toxicosis, liver cancer, and morbidity in children suffering from kwashiorkor (Miller, 1996; Widstrom, 1996; Gong *et al.*, Chapter 6). The consumption of fumonisin-contaminated maize foods also has been associated with outbreaks of esophageal cancer (Rheeder *et al*., 1992; Thiel *et al*., 1992) and neural tube birth defects in humans (Stack, 1998). Some reports suggest that various processing methods including roasting, boiling, frying, baking and fermenting, may not effectively eliminate either aflatoxin (Widstrom, 1996) or fumonisin (Hendrich *et al.*, 1993**;** Voss *et al*., 1996; Fandohan *et al.*, Chapter 26) from contaminated maize food products.

Several approaches have been proposed for reducing aflatoxin (Widstrom, 1996) and fumonisin (Norred *et al.*, 1991; Hendrich *et al.*, 1993; Voss *et al.*, 1996; Katta *et al*., 1997) levels in unprocessed grain and processed maize-based food products. One promising strategy is to grow maize cultivars that are resistant to *Aspergillus* and *Fusarium* ear rot and accumulate less mycotoxin in the grain (Widstrom, 1996; Kleinschmidt *et al.*, 2005). Moderate to high levels of resistance to *A. flavus* (Gorman and Kang, 1991; Brown *et al.*, 1999, 2001) and *F. verticillioides* (Widstrom, 1996; Clements *et al*., 2004) are known in maize. The use of these types of resistance in combination with appropriate cultural practices can reduce the total amount of mycotoxin accumulation. Preharvest host resistance to *A. flavus and F. verticillioides* is a simple and economical technology that leaves no harmful residue in food or the environment, and can be applied over a broad range of environmental and socioeconomic conditions. Although several maize genotypes that accumulate only low levels of aflatoxin and fumonisin have been identified, most of these lines lack desirable agronomic backgrounds, with adaptation problems and the relatively high levels of toxin that can still accumulate sufficing to prevent commercial deployment (Gorman and Kang, 1991; Brown *et al*., 1999; Clements *et al.*, 2004; Brooks *et al.*, 2005; Kleinschmidt *et al.*, 2005). Thus, there is a need to develop maize germplasm with desirable agronomic traits and reduced levels of mycotoxin contamination. This chapter evaluates the breeding strategies currently used at IITA to develop germplasm resistant to infection by *A. flavus and F. verticillioides*, which includes: (i) field selection for reduced ear rot infection, (ii) breeding for reduced aflatoxin accumulation, and (iii) screening elite germplasm for resistance to specific fungi.

Field selection for reduced ear rot infection

Selecting germplasm resistant to ear rots has a crucial role in IITA's strategy for developing maize germplasm targeted to the forest zones and mid-altitude regions of Central and West Africa. Every year, early generation and advanced breeding lines developed from diverse sources of germplasm, as well as varieties and hybrids, are screened in "hot spot" locations representative of the production zones where ear rot occurs regularly at high levels. Promis-

ing materials are selected following visual assessment for reduced levels of ear rot. Environmental factors affect naturally occurring ear rot severity, so the breeding materials are evaluated repeatedly at the "hot spot" locations and at different stages of development to identify materials with reasonable levels of resistance to kernel and ear rot infection. The breeding materials also have been evaluated for tight husk cover, which is the first line of defense against infection by fungi causing ear rot (Widstrom, 1996). The detection of high concentrations of mycotoxins in maize genotypes exhibiting minimal ear rot infection symptoms (Clements *et al.*, 2004; Kleinschmidt *et al.*, 2005) emphasizes the need to select directly for maize germplasm with reduced mycotoxin accumulation.

Breeding for reduced aflatoxin accumulation

Screening for reduced aflatoxin accumulation in parental materials

Effective, reliable, rapid screening techniques are indispensable for breeding for lower levels of aflatoxin accumulation in maize (Gorman and Kang, 1991; Brown *et al.,* 1999). Brown *et al.* (1995) developed a rapid laboratory-based kernel-screening assay that creates high uniform levels of infection and aflatoxin production and enables the differentiation of maize genotypes that accumulate low and high levels of aflatoxins. This assay provides a consistent ranking of maize genotypes in different tests and the results are correlated with results obtained in field trials (Brown *et al.*, 1995).

Seventy-six inbred lines from IITA with moderate to high levels of field resistance to ear rots in the forest zone and the mid-altitudes were evaluated at the Southern Regional Research Center (SRRC)-USDA-ARS laboratory with the kernel screening assay (Brown *et al.*, 2001). Eighteen of these inbred lines had aflatoxin levels that were as low as or lower than those of the best lines from the United States. Further studies with some lines found that the protein profiles of the IITA lines were different from those developed in the United States, suggesting that the IITA lines and those from the United States carry different alleles for the reduction of aflatoxin accumulation. By assessing fungal growth on selected lines, a unique line with low aflatoxin accumulation, but a high level of fungal growth was identified (Brown *et al.*, 2001). This result suggests that toxin accumulation may be inhibited directly in addition to being related to the amount of fungal infection present. The lowest toxin accumulating lines from IITA were crossed with similar genotypes from the United States in a collaborative breeding project (Brown *et al.*, 2003). This strategy increases the probability of developing inbred lines with good agronomic traits that accumulates less toxin than do the currently available commercial lines.

Genetics of resistance to aflatoxin accumulation

The mode of inheritance of resistance to *Aspergillus* ear rot and reduced aflatoxin accumulation in maize grain is not settled. In some studies resistance to ear rot and lower aflatoxin accumulation levels are quantitatively inherited (Walker and White, 2001), with additive gene effects playing a major role the inheritance of resistance (Norred *et al.*, 1991; Miller, 1996; Naidoo *et al*., 2002). In other studies, dominance has a greater effect on the reduction of aflatoxin accumulation than does additive gene action (Campbell *et al.*, 1997; Campbell and White, 1995; Maupin *et al.*, 2003; Busboom and White, 2004). Broad-sense herita-

Table 1. Mean and range of values (ng/g) for each group of inbred lines evaluated with the kernel screening assay in 2003 and 2004.

Group	Number of lines	Range	Mean ± Standard error
Lines derived from backcrosses			
I	14	6 – 6,000	1,700 ± 420
II	6	0 – 5,200	2,000 ± 670
III	10	360 – 5,100	1,500 ± 530
IV	13	75 – 10,200	1,500 ± 740
V	15	40 – 2,100	930 ± 170
VI	12	150 – 7,900	1,600 ± 660
VII	8	140 – 4,800	2,700 ± 740
Lines derived from tropical × temperate crosses – Set-1			
I	12	890 – 15,000	6,500 ± 1,300
II	10	740 – 14,000	6,500 ± 1,400
III	11	3,000 – 9,100	6,000 ± 530
IV	9	0 – 4,400	1,900 ± 450
V	9	80 – 3,700	2,200 ± 380
VI	9	500 – 9,300	5,300 ± 880
VII	9	390 – 3,600	1,500 ± 310
VIII	7	250 – 5,600	3,300 ± 740
IX	11	230 – 4,600	1,300 ± 430
Lines derived from tropical × temperate crosses – Set-2			
I	9	990 – 14,000	7,900 ± 1,500
II	11	130 – 7,400	2,100 ± 790
III	11	460 – 6,600	2,500 ± 660
IV	11	650 – 9,500	5,000 ± 830
V	12	700 – 12,000	4,200 ± 1,300

bility estimates for both ear rot resistance and lowered aflatoxin levels are moderate to high (Norred *et al.*, 1991; Maupin *et al.*, 2003), suggesting that selection for resistance should be feasible. Significant progress has been made in identifying sources of resistance and understanding their genetic basis, but neither the germplasm nor the genetic information has been used to breed commercially useful maize that accumulates less aflatoxin. The trait's complex of inheritance, the erratic nature of field infection by *A. flavus*, and the year-to-year variability in aflatoxin levels have limited transfer of these traits to elite maize inbred lines since selections made under field conditions often could not be relied on (Gorman and Kang, 1991; Brooks *et al.*, 2005). The development of new efficient tools for screening maize genotypes in both field and laboratory settings increased the number of breeding strategies available for developing resistant maize germplasm that accumulates less toxin (Brown *et al.*, 2003).

Creating populations and developing lines

Both pedigree and backcross breeding methods have been used to develop maize lines with new combinations of agronomic traits and resistance to diseases. Five elite tropical inbred lines from IITA

Table 2. Mean aflatoxin values (ng/g) for a group of inbred lines derived from backcrosses evaluated with the kernel screening assay.

Line	Pedigree	Aflatoxin
L01	P3142 (US Susceptible check)	2,100 a[a]
L02	(MP420 × 4001 × MP420)-2-2-3-1-B	1,700 a
L03	(MP420 × 4001 × MP420)-2-2-3-3-B	1,600 a
L04	MP420 (Recurrent parent)	1,500 a
L05	MP420 × 9450 × MP420-3-1-1-2-B	1,400 ab
L06	MP420 × 9450 × MP420-3-1-1-3-B	1,400 ab
L07	(MP420 × 4001 × MP420)-2-2-3-2-B	1,200 ab
L08	(MP420 × 4001 × MP420)-3-1-3-2-B	800 abc
L09	(MP420 × 4001 × MP420)-3-1-3-1-B	770 abc
L10	(MP420 × 4001 × MP420)-2-2-3-4-B	640 bcd
L11	(MP420 × 4001 × MP420)-3-1-2-1-B	320 cde
L12	MP420 × 9450 × MP420-3-1-1-4-B	300 cde
L13	MI82 (US resistant check)	110 def
L14	(MP420 × 4001 × MP420)-2-1-1-1-B	63 def
L15	(MP420 × 4001 × MP420)-3-1-2-2-B	43 fg

[a]Means followed by the same letter are not significantly different based on the least significant difference test (p = 0.05).

(Babangoyo, KU1414-SR, 1368, 4001, and 9450) were crossed to three or four selected genotypes from the United States (B73×Tex6, C12, GT-MAS:gk, MI82, MO 17×Tex6, MP420, OH516, and T115) that accumulated low levels of aflatoxin (Brown *et al.*, 1995) to form 16 F_1 crosses. Each F_1 cross was crossed with the parental genotype from the United States as a recurrent parent to generate 16 backcross (BC_1) populations. In addition, seven elite IITA inbred lines (Babangoyo, KU1414-SR, 1368, 4001, 5012, 9071, and 9450) were crossed to two or three of the same set of US genotypes to develop 16 F_1 crosses.

Measuring the amount of aflatoxin produced by *A. flavus* in maize is both tedious and expensive, so we could not evaluate aflatoxin production in a large number of individual plants derived from each of the many segregating populations. Aflatoxin production was not assessed until homozygous lines (S_5) were developed following selection for agronomic traits and resistance to foliar diseases during the earlier stages of inbreeding. From 2000 to 2002, self-pollinated ears were selected from each row to develop lines from each BC_1 or F_1 cross. At each stage of inbreeding, visual selection within and among lines was made on the basis of synchrony between pollen shed and silking, low ear placement, well-filled ears and resistance to lodging and foliar diseases, including *Puccinia polysora*, *Bipolaris maydis* and *Curvularia lunata*, under naturally occurring disease pressure at Ibadan, Nigeria. Sixty-five S_5 lines were developed from the backcross populations and 144 S_5 lines were derived from F_1 crosses to be screened with the kernel screening assay (Brown *et al.*, 1995).

Screening lines derived from populations with the kernel screening assay

The 57 S_5 lines derived from the backcross populations were divided into seven groups, each containing three to eleven S_5 lines, the recurrent parent and resistant and a susceptible inbred checks. These groups were screened for reduced aflatoxin accumulation (Brown *et al.*, 1995). The maize inbred lines within each group exhibited a broad range in aflatoxin

Table 3. Mean aflatoxin values (ng/g) for a group of inbred lines derived from tropical × temperate crosses evaluated with the kernel screening assay.

Line	Pedigree	Aflatoxin
TL01	1368	7,400 a[a]
TL02	P3142 (US Susceptible check)	7,200 a
TL03	1368 × MI82-13-1-1-1-B	2,100 ab
TL04	1368 × MI82-23-1-1-2-B	1,500 b
TL05	1368 × MI82-19-4-1-1-B	1,200 bc
TL06	1368 ×MI82-11-2-1-1-B	1,200 bc
TL07	1368 × MI82-23-1-1-1-B	770 cd
TL08	MI82 (US resistant check)	670 cd
TL09	1368 × MI82-23-1-1-3-B	580 cd
TL10	1368 × MI82-11-1-1-1-B	560 cd
TL11	1368 × MI82-17-1-1-1-B	130 d

[a]Means followed by the same letter are not significantly different based on the least significant difference test ($p = 0.05$).

accumulation (Table 1). Inbred lines derived from backcrosses included in Group V and those derived from tropical × temperate crosses included in Group II, which were reported in Table 1, were chosen as examples to provide highlights of the results of the screening assay presented in Tables 2 and 3, respectively. Among the lines included in Group V, five S_5 lines (L10 – L15) accumulated significantly ($p < 0.05$) less aflatoxin than did the recurrent parent from the United States, MP420. L11, L12, L14, and L15 did not differ significantly in aflatoxin accumulation from the resistant US inbred check, MI82. Two pairs of S_5 lines (L02 and L10, and L05 and L12), which were advanced to the S_3 stage of inbreeding from the same single plant, differed significantly ($p < 0.05$) in aflatoxin accumulation (Table 2). Of the 57 S_5 lines evaluated, 23 accumulated significantly ($p < 0.05$) less aflatoxin than did their respective recurrent parent. Some of these lines also had aflatoxin contamination levels similar to or lower than the resistant inbred check from the United States, MI82.

The S_5 lines derived from the F_1 crosses also were divided into groups and screened for aflatoxin accumulation with the kernel screening assay. Significant ($p < 0.05$) differences in aflatoxin production again were detected amongst the lines within each group (Table 1). All of the S_5 lines (TL04 to TL011) differed significantly ($p < 0.05$) in aflatoxin accumulation from the elite tropical parental line, 1368, but not from the resistant inbred check, MI82 (Table 3). We found two pairs of inbred lines (TL04 and TL09 and TL04 and TZ07) that originated from the same single plant at the S_3 stage that had contrasting aflatoxin accumulation levels (Table 3). Thirty-two of the 102 S_5 lines evaluated had significantly lower aflatoxin levels than the elite tropical inbred parent. About half of these lines did not differ significantly in aflatoxin accumulation from the inbred check, MI82.

Selection of genetically similar inbred lines for proteome analysis

We defined a pair of inbred lines from the same backcross or F_1 cross, expected to share at least 88% common genetic background, as genetically similar. Seven pairs of S_5 lines derived from backcrosses and ten pairs of lines extracted from F_1 crosses had 88-97% genetic identity but differed significantly in aflatoxin accumulation. Proteomic analyses of kernel, embryo and

Table 4. Eigenvectors of the first three principal component axes (PC1, PC2 and PC3) for the various traits of 54 maize inbred lines grown at Saminaka and Ikenne in Nigeria in 2004.

Trait	PC1	PC2	PC3
Days to silking	0.11	0.10	0.82****
Plant height (cm)	-0.05	0.68****	-0.03
Ear height (cm)	-0.17*	0.65****	0.01
Husk cover (1-5)[a]	0.33****	-0.10	-0.32**
Plant aspect (1-5)[b]	0.41****	0.24*	-0.33**
Ear aspect (1-5)[c]	0.47****	0.06	0.25*
Ear rot (1-5)[d]	0.48****	-0.05	0.18
Lowland leaf blight (1-5)[e]	0.41****	0.17	-0.13
Lowland leaf rust (1-5)[e]	0.25***	0.00	0.00
Variance	0.39	0.21	0.14

*, ***, **** Significantly different from zero at $p < 0.05$, $p < 0.001$ and $p < 0.0001$ levels, respectively

[a]Husk cover: A scale of 1 to 5, where 1 = very tight husk extending well beyond the ear tip and 5 = exposed ear tip.

[b]Plant aspect (1-5): 1 = excellent overall phenotypic appeal and 5 = poor overall phenotypic appeal.

[c]Ear aspect (1-5): 1 = clean, uniform, large, and well-filled ears and 5 = rotten, variable, small and partially filled ears.

[d]Ear rot (1-5): 1 = little or no visible ear rot and 5 = extensive visible ear rot.

[e]Disease scores recorded at 26 days after mid-silking on a 1-5 scale, where 1 = no visible infection and 5 = severe infection on all leaves.

endosperm proteins associated with lower levels of accumulated aflatoxin has relied on side-by-side comparisons of lines with genotypes with different genetic backgrounds (Chen *et al.,* 2004, *a*,*b*). The identification of genetically similar lines differing significantly in aflatoxin accumulation should enable the identification of candidate genes underlying resistance to *A. flavus* infection and/or reduction of aflatoxin production without the confounding effects that result when lines of diverse genetic background are compared (Brown *et al.*, 2003). Comparing pairs of genetically similar lines in a proteome analysis, has thus far identified several resistance-associated proteins, categorized as stress-related, and a putative regulatory protein (Brown *et al.*, 2003). The expression of stress-related proteins may enable a plant to defend against fungal invasion under stress conditions. Extensive analysis of kernel endosperm proteins of several pairs of genetically similar lines emanating from our collaborative breeding project are described elsewhere (Brown *et al.*, 2007). This may facilitate identification of potential markers for rapid screening of genetic materials in a breeding program. Proteins identified in such a screen may be useful markers for rapidly screening genetic materials in a breeding program.

Evaluating agronomic performance of selected lines

Inbred lines that accumulate less aflatoxin need to be evaluated for agronomic performance as inbred lines *per se* and as parents of hybrids to determine their usefulness in a breeding program. We screened 54 inbred lines for aflatoxin accumulation under field conditions at Saminaka and Ikenne, Nigeria in 2004. Principal component analysis was used to evaluate the field data and to assess the performance of the new inbred lines on the basis of their agronomic traits. The first principal component axis (PC1) accounted for nearly 40% of the total variation in the data set (Table 4). A high PC1 score was associated with significant reduction in ear height, poor husk cover, plant aspect and ear aspect scores, and increased ear rot, leaf blight and leaf rust infec-

Table 5. Mean aflatoxin values (ng/g) of inbred maize lines selected for low aflatoxin production in the kernel screening assay (KSA) and evaluated under artificial inoculation in the field at Ibadan, Nigeria in 2003 and 2004.

Inbred line	Field trials			KSA
	2003	2004	Mean	
1368	1240	260	750	78
1823	370	96	230	39
TZMI102	1700	130	930	21
TZM104	310	310	310	270
TZMI502	240	93	170	70
Mean ± S.E.	780 ± 290	180 ± 71	440 ± 130	95 ± 43

tions. The second component axis (PC2) explained 21% of the total variation in agronomic traits recorded in this trial and its large scores were associated with taller plants, high ear placement and poor ear aspect scores. The third component axis (PC3) accounted for 14% of the total variation in agronomic traits recorded in this trial and its large scores were associated with a delay in silking, good husk cover and plant aspect scores but with poor ear aspect score. Grain yield of the inbred lines was negatively correlated with PC1 (r = -0.73, $p < 0.0001$) scores but not with PC2 (r = -0.24, p = 0.08) and PC3 (r = -0.12, p = 0.40) scores of the inbred lines. We found some inbred lines with high grain yields and negative PC1 scores, indicating that some of them accumulated less aflatoxin but also had good husk cover, plant aspect and ear aspect scores as well as lower levels of ear rot, leaf blight and leaf rust infections.

Screening elite germplasm for resistance to specific fungi

Evaluating resistant lines under artificial field infection

Field tests of inbred lines identified in the kernel screening assay are critical for identifying the best lines with consistently low levels of aflatoxin accumulation. The ultimate phase in the identification of lines that consistently accumulate less aflatoxins is the exposure of such lines to many populations of *A. flavus* under as wide a range of environmental conditions as possible. Nine inbred lines selected for low levels of aflatoxin production based on the kernel-screening assay were evaluated with artificial inoculation in the field in Nigeria in 2003. Among these lines, 1823, TZMI104 and TZMI502 had low levels of aflatoxin contamination under field conditions (Table 5). These three lines along with 1368 and TZMI102 also were evaluated in 2004. Two of the three inbred lines (1823 and TZMI502) that had low aflatoxin levels in 2003 also had low levels of aflatoxin in 2004, indicating that the laboratory-based kernel screening assay can be used to reliably screen breeding material prior to testing under field conditions (Brown *et al.*, 1999).

Screening elite germplasm for reduced fumonisin production

Mixed infections with *A. flavus* and *F. verticillioides* occur in maize under field conditions. Thus, any strategy to reduce mycotoxin accumulation also should identify sources of resistance to fumonisin accumulation and incorporate them into adapted germplasm. IITA is

screening elite maize germplasm for reduced fumonisin accumulation. A replicated field trial of 58 elite inbred lines was conducted at Ibadan, Nigeria in 2003 and 2004, in which emerging silks were artificially inoculated with a spore suspension of an isolate of *F. verticillioides*. At Ikenne in 2003 the response to natural infection was evaluated. The inbred lines differed markedly in fumonisin accumulation at both locations and in both seasons. At Ibadan, mean fumonisin concentration in the grain ranged from 1.1 to 130 μg/g in 2003 and from 0.2 to 99 μg/g in 2004. At Ikenne, mean fumonisin concentration in the grain ranged from 0 to 120 μg/g in 2003. The number of lines at Ibadan with ≤ 5.0 μg/g fumonisin was nine in 2003 and 21 in 2004. Thirty-five inbred lines had < 5 μg/g fumonisin in the grain at Ikenne in 2003. Three inbred lines [(1368/S.A. Pub Lines36/1368)-2-2-2-B, KU1414×ICAL 36-1×KU1414-6-1-B and (CIM 116 × TZMi 302 × CIM 116)-2-2-B] and a commercial hybrid (Oba Super I) had < 5 μg/g fumonisin in the grain in both seasons at Ibadan and in the one season at Ikenne. These results re-emphasize the need to conduct multi-location and multi-season evaluations of genetic materials to identify sources of resistance with consistently low levels of fumonisin accumulation.

Conclusions

Maize inbred lines with consistently low aflatoxin levels after repeated evaluation in the laboratory and in the field could be used in the development of hybrids and synthetic varieties that can be deployed in farmers' fields to help reduce mycotoxin contamination. The new inbred lines also could be used to broaden and to diversify the genetic base of resistant germplasm in maize breeding programs. The advances made in identifying genetically similar lines with contrasting aflatoxin levels may enable the characterization of mechanisms responsible for lower levels of aflatoxin accumulation and the identification of candidate genes that underlie resistance to *A. flavus* infection and/or aflatoxin accumulation, access to novel variants, and the development of markers for rapid screening of breeding materials. Promising lines with low fumonisin accumulation also could be used in crosses with inbred lines that accumulate lower levels of aflatoxin to develop new lines with combined resistance to both fungal species and to lower the contamination levels of both of these very important mycotoxins.

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