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Article

Evaluation of Maize Germplasm for Resistance to Aflatoxin Accumulation

W. Brien Henry [1,*], Gary L. Windham [1] and Michael H. Blanco [2]

[1] USDA-ARS, Corn Plant Host Resistance Research Unit, Box 9555, Mississippi State, MS 39762, USA; E-Mail: gary.windham@ars.usda.gov

[2] GEM Coordinator, USDA-ARS, Plant Introduction Research Unit, G212 Agronomy Hall, Iowa State University, Ames, IA 50011, USA; E-Mail: michael.blanco@ars.usda.gov

* Author to whom correspondence should be addressed; E-Mail: brien.henry@ars.usda.gov; Tel.: +1-662-325-8023; Fax: +1-662-325-8441.

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Abstract: Aflatoxin contamination of maize grain threatens human food and animal feed safety. Breeding for reduced grain aflatoxin accumulation is one of the best strategies presently available to lower grain aflatoxin accumulation. Previously identified sources of germplasm with reduced grain aflatoxin accumulation are excessively tall and late maturing. The objective of this research was to screen germplasm and identify potential sources of aflatoxin resistance. KO679Y and CUBA117:S15-101-001-B-B-B-B inbreds were evaluated for aflatoxin accumulation alongside resistant and susceptible checks with both performing well. These two lines were also evaluated in various crosses. KO679Y performed especially well in crosses with Mp494 and Mp717, resulting in low ear rot and very low aflatoxin levels, but not well in other crosses. A breeding cross including CUBA117:S15-101-001-B-B-B-B as a parent accumulated low levels of aflatoxin both years it was evaluated. Lines resulting from these crosses are being advanced for further evaluation and improvement. KO679Y and CUBA117:S15-101-001-B-B-B-B may prove useful for breeders seeking germplasm sources for ear rot and mycotoxin reduction, especially KO679Y which matures a week earlier and is approximately 25% shorter than current lines resistant to grain aflatoxin accumulation.

Keywords: *Zea mays*; *Aspergillus flavus*; aflatoxin; inbred; KO679Y; hybrid; single cross

1. Introduction

Aspergillus flavus produces aflatoxin which is one of the most problematic mycotoxins [1,2]. Mycotoxin contamination of maize grain threatens both human food and animal feed safety [3–6]. If aflatoxin levels exceed legal limits, farmers in the United States may be unable to market their grain thereby suffering economic losses. Humans with limited food options, such as those of subsistence farmers in developing countries, often must consume aflatoxin-contaminated grain resulting in an increased incidence of liver cancer. Hepatocelluar carcinoma occurs up to 32 times more frequently in developing countries than in developed countries [7]. Chronic exposure of humans to aflatoxin in food results in suppressed growth rates of children and decreased life expectancies. Acute exposure can result in toxicosis and rapid death of animals that consume toxin laden grain.

Breeding maize (*Zea mays* L.) for resistance to *A. flavus* infection and subsequent grain aflatoxin accumulation is considered to be one of the best and most cost effective strategies currently available to reduce aflatoxin losses [8]. Since the early 1970s breeding efforts to lower grain aflatoxin accumulation have resulted in the release of four Mississippi lines Mp313E, Mp420, Mp715 and Mp717 [9–11]. Two recently released lines, Mp718 and Mp719 were derived from an Mp715 by Va35 cross resulting in resistant material that is 13 days earlier than the resistance source, Mp715 [12]. Additional maize lines that limit aflatoxin accumulation have been released by researchers in the Southeast United States. The USDA-ARS at Tifton, GA released GT603 with improved agronomic characteristics compared with other resistance sources previously released [13]. Texas A&M researchers released three lines Tx736, Tx739 and Tx740 with both favorable agronomic and reduced toxin accumulation compared with commercial lines [14]. Additional sources of resistance are currently being sought using conventional breeding, marker assisted breeding, and association mapping to identify and develop lines by USDA-ARS Mississippi State, MS. Preliminary marker studies suggest that resistance to grain aflatoxin accumulation is a polygenic trait with multiple quantitative trait loci (QTL) of varying significance within a given line [15]. QTL's also differ among location and significance across resistant inbred lines suggesting the existence of multiple mechanisms for imparting reduced grain aflatoxin accumulation.

Evaluating new germplasm in replicated field trials with known resistant and susceptible checks can assist in the identification of new, and ideally robust, sources of mycotoxin resistance. This technique has been used to screen potential new sources of resistance provided by the Germplasm Enhancement of Maize (GEM) Project. The GEM Project is a collaborative effort of USDA-ARS, universities, private industry, international agricultural research centers and non-governmental organizations (NGO) to broaden the germplasm base of maize [16,17]. The GEM Project's goal is the development of adapted germplasm for the US Corn Belt utilizing tropical and temperate exotic germplasm. Li *et al*. [18] reported that substantial variability existed among maize breeding crosses for aflatoxin resistance from germplasm evaluated from the GEM Project. It was suggested that the most resistant breeding crosses be used to develop new inbred lines. The objectives of this study were (1) to evaluate aflatoxin accumulation in inbred lines and (2) to evaluate aflatoxin accumulation in breeding crosses and single-cross hybrids constructed with potential sources of resistance.

2. Results and Discussion

Maize inbred lines, hybrids and crosses were screened for reduced aflatoxin accumulation in 2009–2011. In 2009, inbred line KO679Y was numerically the third lowest of the 30 lines tested, and statistically the same as the four resistant checks, entries 21, 22, 25, and 26 (Table 1).

Table 1. Aflatoxin evaluation of corn inbreds in 2009.

Entry	Pedigree	GEM Code	Aflatoxin[†]		DTM[§]	
			ng g^{-1}			
16	FS8B(T):N11a08c-030-002-B-B	GEMN-0179	7103	a[‡]	62.7	ij
28	NEI9008:S17c21-091-001-B-B	GEM EXP	2629	ab	64.7	g–j
24	GA209	.	2556	ab	72.3	b
27	BR51721:N2012-164-002-B-B-SIB	GEMN-0157	2482	ab	65.7	f–j
23	SC212M	.	2476	ab	73.0	b
2	2132-03_DK888_S11_F2S4_9187-Blk22/00-Sib-B-B	GEMS-0016	2255	ab	64.7	g–j
20	BR52060:S0210-147-001-B-B-B	GEMS-0143	2169	ab	66.0	e–i
19	UR11003:S0302-1011-001-B-B-B	GEMS-0118	2086	a–c	63.3	h–j
4	BR51675:N0620-033-001-B-B-B	GEMN-0140	2063	a–d	64.0	h–j
14	1895-001/98_DKXL370AN11F2S3_7521-29-B-B	GEMN-0133	1660	a–e	68.0	c–g
30	UR11003:S0302-937-001-B-B-Sib	GEMS-0100	1595	a–e	62.0	j
5	AR16026:S17-010-001	GEMS-0061	1272	b–f	69.3	b–f
17	DK888:N11a08a-395-001-B-B	GEMN-0177	980	b–g	69.7	b–e
15	CHIS740:S1411a-783-002-B-B-Sib	GEMS-0091	859	b–g	62.7	ij
29	CH05015:N15-003-001-B-B-SIB-SIB	GEMN-0071	654	b–h	67.0	d–h
12	2258-03_XL380_S11_F2S4_71/97_Bulk/98-Sib-B-B-B-B	GEMS-0030	596	b–h	70.3	b–d
1	CUBA164:S1517-163-001-B-B	GEMS-0074	440	c–i	64.7	g–j
3	2088-01_DK212T_S11_F2S4_9157-Blk29/00-sib-B-B-B	GEMS-0006	425	d–j	68.0	c–g
7	1881-002/98_DKXL370AN11F2S3_7521-05-B-B	GEMS-0128	405	e–j	67.0	d–h
8	1886-003/98_DKXL370AN11F2S3_7521-05-B	GEMN-0132	319	f–k	71.0	bc
6	FS8A(S):S09-43-2	GEMS-0002	318	f–k	62.7	ij
10	1883-002/98_DKXL370AN11F2S3_7521-05-B-B	GEMN-0131	232	g–l	69.7	b–e
22	Mp313E	.	221	g–m	79.5	a
13	CUBA117:S15-101-001-B-B-B-B	GEM EXP	215	h–m	79.0	a
21	Mp717	.	150	h–m	77.0	a
9	1883-001/98_DKXL370AN11F2S3_7521-05-B-B	GEMN-0130	104	i–m	70.3	b–d
26	Mp494	.	87	j–m	80.0	a
18	KO679Y	PI 591017	75	k–m	70.3	b–d
11	DKXL380:N11a18-059-001	GEM EXP	58	l–m	68.0	c–g
25	Mp317	.	45	m	79.0	a

[†] Means for aflatoxin concentration were transformed [ln (y + 1)] before statistical analysis, and tests for significance were performed on transformed means prior to conversion back to the original scale; [§] DTM Days to midsilk, number of days from planting until silks emerge on half of the plants in a row; [‡] Means in a column followed by the same letter do not differ at $P < 0.05$ (Fisher's Protected LSD).

Of the four low-aflatoxin accumulating checks that were included in the trial, KO679Y numerically accumulated lower toxin, 75 ng g^{-1}, than three of the entries and was not statistically different from any of them. KO679Y was included in the 2010 inbred aflatoxin evaluation and had the 6th lowest aflatoxin level of the 20 entries, 223 ng g^{-1} (Table 2).

Table 2. Aflatoxin evaluation of corn inbreds in 2010.

Entry	Pedigree	GEM Code	Aflatoxin † ng g^{-1}		DTM §	
17	Mp317	.	.	.	85.0	a
20	SC212M	.	5247	a ‡	81.3	b
10	DKXL380:S08a12-288-001-B-B	GEM EXP	3294	ab	75.0	c–e
19	GA209	.	2155	a–c	78.0	c
8	DKXL380:S08a12-069-001-B-B	GEM EXP	1963	a–c	73.7	ef
9	DKXL380:S08a12-165-001-B-B	GEM EXP	1422	a–d	71.0	f
7	DKXL380:N11a18-059-001-B-B	GEM EXP	1243	a–e	73.3	ef
13	FS8B(T):N11a-087-001-B-B-SIB-B	GEMN-0097	1092	a–e	71.0	f
15	DKB844:S1601-073-001-B-B-B-B-B	GEMS-0115	817	a–e	76.0	c–e
3	DKXL380:S11-B-004-003-B-B-Sib-B-B-B-B-B	GEMS-0030	653	b–e	76.0	c–e
5	AR16026:S17-010-001-B-B-B	GEMS-0061	539	b–e	75.0	c–e
16	Ki21	.	401	c–e	81.3	b
6	DKXL370A:N11-B-005-010-B-B	GEMN-0130	333	c–e	76.0	c–e
11	DKXL380:S08a12-294-001-B-B	GEM EXP	254	d–e	74.7	de
14	KO679Y	PI 591017	223	de	77.0	cd
12	CUBA117:S15-101-001-B-B-B-B-B-B-B	GEM EXP	209	ef	73.3	ef
2	DKXL370A:S11-B-025-001-B-B-Sib-B-B	GEMS-0027	207	ef	71.0	f
18	Mp717	.	33	fg	82.0	ab
4	DKXL380:S11-B-029-001-B-B-Sib-B-B-B	GEMS-0032	28	g	83.0	ab
1	DKXL370A:S11-B-025-002-B-B-Sib	GEMS-0028	24	g	73.7	ef

† Means for aflatoxin concentration were transformed [ln (y + 1)] before statistical analysis, and tests for significance were performed on transformed means prior to conversion back to the original scale; § DTM Days to midsilk, number of days from planting until silks emerge on half of the plants in a row; ‡ Means in a column followed by the same letter do not differ at $P < 0.05$ (Fisher's Protected LSD).

Another GEM experimental line from Cuba, CUBA117:S15-101-001-B-B-B-B, fared equally well with 215 and 209 ng g^{-1} aflatoxin accumulation in 2009 and 2010, respectively. This line was statistically similar to all the resistant checks both years (Table 1, Table 2).

Four of the entries in the 2010 inbred evaluation (DKXL entries 1–4) were identified as low grain aflatoxin accumulators in prior evaluations [19], and for the most part they performed well in 2010 (Table 2). Mp317 and Mp717 were included as resistant checks, but Mp317 did not produce ears suitable for inoculation or harvest because of the heat and drought.

In addition to favorable toxin data, KO679Y was significantly earlier than the resistant checks included in the inbred evaluations. Compared with the resistant checks, KO679Y was approximately 10 days earlier in 2009 and 7 days earlier in 2010 (Table 1, Table 2).

Approximately 150 breeding crosses and 50 hybrid crosses were evaluated in 2008 for aflatoxin accumulation (data not shown), and several of the best entries were evaluated for aflatoxin accumulation again in 2009 (Table 3).

In addition to repeated evaluation of some of the better performing crosses from 2008, additional breeding crosses containing DKXL or CUBA117:S15-101-001-B-B-B-B germplasm were also included in the 2009 trial (Table 3). Of the breeding crosses identified with low aflatoxin accumulation in 2008 and retested in 2009, entries 32 through 36 fared well with toxin levels at or below 100 ng g^{-1}. Conversely, entries 31, 37, and 38 were all above 300 ng g^{-1} (Table 3).

Table 3. Aflatoxin evaluation of corn hybrids and breeding crosses in 2009.

Entry	Pedigree/GEM code	Aflatoxin †	
		ng g^{-1}	
45	CML323:N1550	1556	a ‡
47	NEI9004:N0803	1043	ab
16	GEMS-0073/GEMN-0132	829	a–c
13	GEMS-0016/GEMN-0140	665	a–d
30	GEMN-0095/LH200	608	a–d
40	(GEMS-0002/GEMS-0003)-B	590	a–e
38	CHIS462:S0405	556	a–e
31	CHIS462:S04	528	a–e
44	GUAT209:N11c50	503	a–f
10	GEMS-0150/GEMN/0140	488	a–f
29	GEMN-0094/LH200	473	a–g
8	GEMS-0116/GEMN-0132	459	a–h
42	NS1/GEMN-0133	441	a–i
46	TZISTRI112/PHB47	392	a–j
20	GEMN-0133/HC33	365	a–j
7	GEMS-0016/GEMN-0097	361	a–j
1	DKXL380:N11a18-059-001/LH200	340	a–k
37	FS8B(S):S17a	334	a–k
19	GEMS-0091/GEMN-0133	317	a–k
39	(CUBA164:S2012-459-001-B/GEMS-0002)-B	317	a–k
11	GEMS-0162/GEMN-0133	308	a–k
9	GEMS-0115/GEMN-0133	305	a–l
15	GEMS-0061/GEMN-0179	305	a–l
2	DKXL380:N11a18-420-001/LH200	284	a–l
12	GEMS-0116/GEMN-0133	262	a–l
26	GEMS-0175/GEMN-0133	262	a–l
4	DKXL380:N11a18-059-001/HC33	250	b–l
14	GEMS-0016/GEMN-0177	224	b–l
25	GEMS-0115/GEMN-0132	161	c–m
3	GEMS-0074/LH185	153	c–n
5	GEMS-0118/GEMN-0140	151	c–n
43	(S21z/GEMS-0115)-B	148	c–n
17	GEMS-0091/GEMN-0132	112	d–n
35	BR106:T33a/LH132	100	e–n

Table 3. *Cont.*

Entry	Pedigree/GEM code	Aflatoxin †	
		ng g^{-1}	
50	T173 x Mp717	92	f–n
34	CUBA164/NEI9004	91	f–n
18	GEMS-0031/GEMN-0133	79	g–o
36	BR105/NEI9004	79	g–o
28	GEMS-0061/GEMN-0130	77	h–o
27	GEMS-0182/GEMN-0132	74	i–o
21	GEMS-0031/GEMN-0131	72	j–o
33	(UR13085/N28)/Cuba117:S1520-156-001-B	72	j–o
6	GEMS-0143/GEMN-0140	65	j–o
24	CUBA117:S15-101-001-B-B-B-B-B/GEMN/0130	59	k–o
32	(UR13085/N28)/GEMS-0002	57	k–o
41	Ki 14:S21z	51	l–o
23	CUBA117:S15-101-001-B-B-B-B-B/GEMN-0140	28	m–o
22	GEMS-0031/GEMN-0132	26	no
48	Mp313E × Mp717	26	no
49	Mp313E × Mo18w	15	o

† Means for aflatoxin concentration were transformed [ln (y + 1)] before statistical analysis, and tests for significance were performed on transformed means prior to conversion back to the original scale; ‡ Means in a column followed by the same letter do not differ at $P < 0.05$ (Fisher's Protected LSD).

The 2010 evaluation of crosses included four entries with KO679Y in the pedigree (Table 4).

Of the four entries, entry 9, accumulated 163 ng g^{-1} of aflatoxin which was the 7th lowest entry, numerically; however, none were statistically different than the susceptible check. Two entries, 17 and 18, included Mp717 as a parent and generated very low aflatoxin levels with 5 and 34 ng g^{-1}, respectively. This was expected because crosses resulting from Mp717 as a parent usually have lowered grain aflatoxin accumulation. These two entries were included because they are known to have resistance (Table 3). On the contrary, entries 8 and 4 both included Mp717 as a parent, albeit in a lesser percentage, but the grain aflatoxin accumulation was 253 and 430 ng g^{-1}, respectively. It appears that the susceptibility of the other portions of the pedigree may have overwhelmed the resistance contributions of Mp717. This may also have been the case with entry 5, and perhaps with the other entries with KO679Y in this particular evaluation; however, these explanations are speculative and would require additional testing to confirm. KO679Y did not appear to combine well for lowered grain aflatoxin accumulation in any of the four combinations evaluated in 2010 (Table 3).

Entry 10, a breeding cross containing the CUBA117:S15-101-001-B germplasm, performed consistently well in both 2009 and 2010 with 28 and 33 ng g^{-1}, respectively.

Table 4. Aflatoxin evaluation of corn hybrids and breeding crosses in 2010.

Entry	Pedigree	Aflatoxin [†]	
		ng g^{-1}	
1	(KO679Y/GEMS-0115)-B	573	a [‡]
19	SC212M × GA209	515	a
20	T173 × SC212M	487	a
5	((KO679Y/GEMS-0115)/GEMS-0162)	454	a
3	(CUBA117:S15-101-001-B-B-B-B-B/GEMS-0027)-B	433	a
4	((GEMS-0115/Mp717)/GEMS-0162)	430	a
2	(GEMN-0140/GEMN-0130)-B	319	a
15	(UR13085/N28)/Cuba117:S1520-156-001-B-B-B (71-Days, Late)	302	a
12	KO679Y/GEMN-0097	276	ab
8	((GEMS-0115/Mp717)/Ki21)	253	ab
16	Mp317 × Jala-B-B	215	ab
11	CUBA117:S15-101-001-B-B-B-B-B/GEMN-0130	196	ab
6	((87916W/Mp717)/4676A)	174	ab
9	((KO679Y/GEMS-0115)/GEMS-0181)	163	ab
7	((GEMS-0115/Mp717)/GEMS-0061)	144	ab
14	UR13085:S99g99u-B-B-B (61 days, Early)	138	ab
13	CUBA117:S15-101-001-B-B-B-B/GEMN-0130	92	ab
18	Mp317 × Mp717	34	bc
10	CUBA117:S15-101-001-B-B-B-B-B/ GEMN-0140	33	bc
17	Mp313E × Mp717	5	c

[†] Means for aflatoxin concentration were transformed [ln (y + 1)] before statistical analysis, and tests for significance were performed on transformed means prior to conversion back to the original scale; [‡] Means in a column followed by the same letter do not differ at $P < 0.05$ (Fisher's Protected LSD).

The 2011 evaluation of crosses had a wide range of aflatoxin accumulation ranging from 0 to 776 ng g^{-1} (Table 5).

Two of the top three entries included KO679Y as a parent. Entry 7, Mp717 × KO679Y, and entry 8, KO679Y × Mp494, accumulated very low levels of aflatoxin at 1 and 2 ng g^{-1}, respectively. Each of these two entries also had very low ear rot scores (Table 4). Ear rot was scored from 0 to 9 with 0 representing no ear rot and 9 representing an ear fully covered with rot. The mean score for this evaluation was 3.4 and the scores for Mp717 × KO679Y and KO679Y × Mp494 were 0.3 and 1.0, respectively. KO679Y did not reduce ear rot across all of the single-cross hybrid combinations. Entry 1, GEMS-0030 × KO679Y, was the second most susceptible entry with an ear rot score of 6.3 (Table 4). It is of interest that entry 8, the KO679Y × Mp494 cross, had low ear rot scores because, although Mp494 is known for low grain aflatoxin accumulation, it often has significant ear rot both as an inbred and in crosses [20]. The KO679Y × Mp494 cross appears to have overcome the ear rot deficiencies of Mp494, while also maintaining reduced grain aflatoxin accumulation.

Table 5. Aflatoxin evaluation of corn hybrids and breeding crosses in 2011.

Entry	Pedigree	Aflatoxin †		Ear Rot §	
		ng g^{-1}			
18	GA209 × SC212M	776	a ‡	4.0	c–f
12	(GEMS-0115/Mp717)/TZAR101	683	a	5.7	a–d
13	(GEMS-0115/MP717)/GEMS-0115	386	ab	6.0	a–c
17	GEMS-0100 × SC212M	381	ab	4.0	c–f
15	KUI44:N99ap	290	ab	7.3	a
14	KO679Y/(GEMS-0002/GEMS-0003)	174	a-c	4.0	c–f
2	NEI9008:S17c21-091-001-B-B-B × KO679Y	112	a-d	2.0	f–h
3	GEMS-0115 × KO679Y	96	a-d	3.7	d–f
5	KO679Y × Mp317	69	b-e	3.0	e–g
4	CUBA117: S15-101-001-B-B-B-B × KO679Y	62	b-e	3.3	ef
1	GEMS-0030 × KO679Y	58	b-e	6.3	ab
6	KO679Y × Mp313E	53	b-e	4.3	b–d
16	Mp313E × GEMN-0157	28	c-e	2.0	f–h
10	Mp317 × GEMS-0115	24	c-e	3.0	e–g
19	Mp494 × NEI9008:S17c21-091-001-B-B-B	15	d-f	0.3	h
9	Mp317 × NEI9008:S17c21-091-001-B-B-B	13	d-f	4.7	b–d
11	GEMS-0030 × Mp317	9	ef	1.0	gh
8	KO679Y × Mp494	2	fg	1.0	gh
7	Mp717 × KO679Y	1	fg	0.3	h
20	Mp717 × Mp313E	0	g	1.0	gh

† Means for aflatoxin concentration were transformed [ln (y + 1)] before statistical analysis, and tests for significance were performed on transformed means prior to conversion back to the original scale; § Ear Rot Was measured on a 0–9 scale. 0 was an ear free of rot, 9 was an ear completely covered with rot; ‡ Means in a column followed by the same letter do not differ at $P < 0.05$ (Fisher's Protected LSD).

Entry 4 was comprised of two of the more promising potential sources of resistance, inbred lines CUBA117: S15-101-001-B-B-B-B and KO679Y; however, it accumulated 62 ng g^{-1}, a value that suggests only moderate resistance, lower than the susceptible checks, but higher than the best resistant crosses and resistant checks.

Both Mp717 and Mp494 were previously identified resistance sources for reduced grain aflatoxin accumulation and each of these inbred lines has a hard, flinty kernel type that appears to have responded well with respect to ear rot and grain aflatoxin accumulation in crosses with KO679Y. Resistance sources Mp317 and Mp494 also combined well with a GEM line having Thai germplasm in 25% of the pedigree, NEI9008: S17c21-091-001-B-B-B, as entries 19 and 9. These crosses were statistically similar to the two best crosses that included KO679Y as a parent. It is interesting that *per se* the GEM line, NEI9008: S17c21-091-001-B-B-B generated elevated grain aflatoxin levels statistically similar to, and numerically higher than, the susceptible checks GA209 and SC212M (Table 1); however, if crossed to resistant lines the resulting crosses perform very well.

3. Experimental Section

3.1. Planting Dates and Experiments

Multiple experiments to evaluate inbreds, crosses and hybrids for aflatoxin accumulation were conducted at the Rodney Foil Research Facility at Mississippi State University (MSU). The 2009 inbred evaluation was planted on 29 April; the breeding cross evaluation and a second inbred evaluation were planted 14 April, 2010, and the single-cross hybrid evaluation was planted on 10 May 2011. The soil type for the 2009 and 2010 experiments was a Marietta fine sandy loam and for the 2011 experiment was a Leeper silty clay loam.

3.2. Germplasm

Seed for the initial evaluations were received from the GEM Project in 2009. One of the lines that performed well in these trials is KO679Y (PI 591017), a public South African inbred obtained from the GEM Project located at the North Central Regional Plant Introduction Station (NCRPIS) in Ames, IA. This inbred has been noted for its excellent ear quality and has been used by the GEM Project as a potential source of resistance to Fusarium and Diplodia ear rot diseases. It is one among many potential sources of resistance evaluated at the USDA-ARS location at Mississippi State. GEMS-0002 was described as a registered release in 2005 [21], and GEMS-0006, GEMS-0028, and GEMS-0030 were registered in 2006 [19]. Nursery seed increase with agronomic evaluation and inbred aflatoxin screening were conducted that year. Favorable toxin data in 2009 resulted in additional evaluations in 2010, and a request to GEM for additional material to evaluate. Material was received from the GEM Project in 2010 and evaluated alongside other experimental crosses including single-cross resistant and susceptible checks. Based on previous results, KO679Y was used as a parent of 8 additional single cross hybrids which were then evaluated alongside other experimental crosses including single-cross positive and negative checks. Single crosses were made at Mississippi State, MS in 2010 from paired row crosses. Four of these inbreds were older Southern lines evaluated previously for aflatoxin accumulation both as inbreds and in various single-cross hybrid combinations. These lines, Mp717, Mp313E, Mp317 and Mp494, previously exhibited resistance to aflatoxin accumulation [19,22,23]. Across most environments, these four inbred lines are generally tall and late-maturing, and are used as sources of resistance to grain aflatoxin accumulation. The other four inbred lines crossed to KO679Y were selections from the GEM Project. Two of these named inbred entries, GEMS-0030 (low aflatoxin) and GEMS-0115 (good yield), were released previously through GEM in 2003 and 2005 respectively. The other two lines to which KO679Y was crossed were CUBA117: S15-101-001-B-B-B-B and NEI9008: S17c21-091-001-B-B-B which are both experimental GEM lines. The Cuban line exhibited somewhat reduced grain aflatoxin accumulation over multiple years and the NEI9008 (Thai) line did not exhibit resistance *per se*; however, in another diallel experiment (data not shown) and in the 2011 crosses evaluation (Table 4) it combined well with older Southern resistant lines to generate single-cross hybrids with low grain aflatoxin accumulation. These two lines also were provided by the GEM Project. The GEM lines other than KO679Y were derived from self pollination in accordance with GEM protocol using the pedigree breeding method. Phenotypic traits and protocols for breeding and writing pedigrees can be found on the GEM Project website [24].

3.3. Plot size and Management

Entries in the inbred, breeding cross, and single-cross hybrid evaluations at MSU were planted in 5.1 m long single-row plots, with rows spaced 0.97 m apart, and thinned to 20 plants per row in a randomized complete block with three replicates. Fertilizer and herbicides were applied in accordance with standard local production practices and at no time throughout the season was weed pressure or fertility limiting for crop growth. All years experienced periodic drought conditions and supplemental furrow irrigation was applied once in 2010 and multiple times in 2009 and 2011, to assure that there would be enough grain to harvest and evaluate.

3.4. Fungal Inoculum and Aflatoxin

A. flavus isolate NRRL 3357 has produced high levels of aflatoxin in maize grain in prior studies [25–27] and was used to increase the inoculum in these experiments. Inoculum was increased according to Windham *et al*. [26]. Briefly, *A. flavus* was grown on sterile maize cob grits (size 2040, Grit-O-Cobs®, The Andersons, Maumee, OH 43537, USA) in 500-ml flasks. Each contained 50 g of grits and 100 mL of sterile, distilled water, and was incubated at 28 °C for 3 weeks. Conidia in each flask were washed from the grits using 500 mL sterile distilled water containing 0.1% Tween 20 per liter and filtered through four layers of sterile cheesecloth. A tree marking gun was used to deliver a 3.4-mL spore suspension containing 3×10^6 *A. flavus* conidia at 7 days after midsilk. The solution was injected underneath the husks near the middle of the ear.

Ears were harvested approximately 60 days following inoculation and placed in a forced air drier at 38 °C for 5 to 7 days. Following drying, the ears from each plot were bulked together, shelled, mixed thoroughly and ground with a Romer Mill (Union, MO). Aflatoxin concentration was determined with the Vicam Aflatest (Watertown, MA) which detects aflatoxin levels as low as 1 ng g^{-1}.

3.5. Statistical Analysis

All statistical analyses were conducted using the SAS software package (version 8.2; SAS Institute Inc., Cary, NC, USA). Logarithmic transformation [log (y + 1)] was used on all aflatoxin data to stabilize the variance. Data were analyzed with the PROC GLM procedure and means were separated using Fisher's protected least significant difference test at $P = 0.05$. Aflatoxin data are reported as geometric means (antilog of the logarithmic mean).

4. Conclusions

In summary, results show that inbred lines KO679Y and Cuba117:S15-101-001-B-B-B-B are promising sources of reduced grain aflatoxin accumulation that should be further evaluated in replicated field trials over multiple years. CUBA117:S15-101-001-B-B-B-B in one of the breeding cross combinations produced very low grain aflatoxin accumulation in 2009 and 2010. This line is under further development in our breeding program. Because there is a shortage of breeding material for reduced grain aflatoxin accumulation, it is helpful to plant breeders to identify and document new sources of germplasm. This is particularly true throughout the southern US, and tropical regions such as Africa where aflatoxin is a serious problem. In addition to being earlier than presently available

sources of aflatoxin resistance, KO679Y is also 25% shorter than other currently available inbred lines. These favorable agronomic characteristics increase the likelihood of KO679Y being used in future breeding programs. Inbred toxin accumulation values for KO679Y compared to known checks were very low in 2009 and moderately low in 2010. Crosses including both KO679Y and CUBA117:S15-101-001-B-B-B-B as parents varied in aflatoxin accumulation. KO679Y appears to combine well with Mp717 and Mp494 (for reduced aflatoxin) both of which are late and tall and have hard, flinty, yellow-orange kernel types. Advancement of the KO679Y × Mp494 cross is underway in our winter nursery. Should these and other KO679Y crosses perform well in upcoming evaluations, it would be worth considering generating a mapping population and identifying specific QTL in KO679Y associated with reduced grain aflatoxin accumulation.

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