Registration of Six Tropical Maize Germplasm Lines with Resistance to Aflatoxin Contamination

Abebe Menkir,* Robert L. Brown, Ranajit Bandyopadhyay, and Thomas E. Cleveland

ABSTRACT

Six tropical maize (*Zea mays* L.) germplasm lines, TZAR101 (Reg. No. GP-568, PI 654048), TZAR102 (Reg. No. GP-569, PI 654049), TZAR103 (Reg. No. GP-570, PI 654050), TZAR104 (Reg. No. GP-571, PI 654051), TZAR105 (Reg. No. GP-572, PI 654052), and TZAR106 (Reg. No. GP-573, PI 654053), with resistance to aflatoxin contamination were developed by the International Institute of Tropical Agriculture through a collaborative breeding project with Southern Regional Research Center of the USDA-ARS. The lines were derived from biparental crosses and backcross populations involving aflatoxin-resistant tropical elite and temperate inbred lines as parents. These lines had aflatoxin levels similar to or lower than a resistant U.S. inbred check, MI82, in both preliminary and confirmation tests conducted in the laboratory using a kernel-based screening assay. Further field tests of the six lines under artificial inoculation with an African strain of *Aspergillus flavus* in Nigeria revealed that these lines had lower levels of aflatoxin compared with elite tropical commercial inbred lines used as checks. These lines also had good agronomic traits and resistance to important diseases in the lowlands, including southern corn leaf blight [caused by *Bipolaris maydis* (Nisikado & Miyake) Shoemaker], southern corn rust (caused by *Puccinia polysora* Underw.), and ear rot.

The International Institute of Tropical Agriculture (IITA) has developed six maize (*Zea mays* L.) germplasm lines, TZAR101 (Reg. No. GP-568, PI 654048), TZAR102 (Reg. No. GP-569, PI 654049), TZAR103 (Reg. No. GP-570, PI 654050), TZAR104 (Reg. No. GP-571, PI 654051), TZAR105 (Reg. No. GP-572, PI 654052), and TZAR106 (Reg. No. GP-573, PI 654053), with resistance to aflatoxin contamination and adapted to the lowlands. Ear rot–causing fungi, including *Aspergillus*, are common in maize in west and central Africa. *Aspergillus flavus* can contaminate the grain with aflatoxins that pose a serious potential health hazard to humans in this part of Africa. The International Institute of Tropical Agriculture has a collaborative breeding project with the Southern Regional Research Center (SRRC) of the USDA-ARS located in New Orleans, LA, to develop maize germplasm with resistance to aflatoxin contamination (Menkir et al., 2006). The six inbred lines selected for resistance to aflatoxin contamination were developed through this collaborative project. These lines also have good levels of resistance to southern corn leaf blight [caused by *Bipolaris maydis* (Nisikado & Miyake) Shoemaker] and southern corn rust (caused by *Puccinia polysora* Underw.), and they are presently at the S_8 to S_{10} stages of inbreeding.

A. Menkir and R. Bandyopadhyay, International Institute of Tropical Agriculture, Oyo Rd., PMB 5320, Ibadan, Nigeria; R.L. Brown and T.E. Cleveland, USDA-ARS, Southern Regional Research Center, New Orleans, LA 70179. Registration by CSSA. Received 16 Jan. 2008. *Corresponding author (a.menkir@cgiar.org).

Abbreviations: IITA, International Institute of Tropical Agriculture; KSA, kernel-screening assay; SRRC, Southern Regional Research Center; TLC, thin-layer chromatography.

Published in the Journal of Plant Registrations 2:xx–xx (2008).
doi: 10.3198/jpr2008.01.0028crg

677 S. Segoe Rd., Madison, WI 53711 USA

Methods

Line Development

The six inbred lines resistant to aflatoxin contamination were derived from biparental crosses and backcross populations involving tropical elite inbred lines (1368, 4001, and KU1414-SR) from IITA (Kim et al., 1987) with some levels of resistance to aflatoxin production (Brown et al., 2001) and inbred lines from the United States (GT-MAS:gk, MI82, and Mp420) with proven resistance to aflatoxin contamination (Brown et al., 1993, 1995; McMillian et al., 1993; Scott and Zummo, 1992) as parents. TZAR101 was derived from a cross of 1368 to GT-MAS:gk, while TZAR102 and TZAR103 were extracted from a cross of the same tropical inbred line (1368) to MI82 (Table 1). TZAR104 was extracted from a backcross involving GT-MAS:gk as a recurrent parent and KU1414-SR as a nonrecurrent parent. TZAR105 and TZAR106 were developed from a backcross involving Mp420 as a recurrent parent and 4001 as a nonrecurrent parent. TZAR102 and

Table 1. Kernel color and texture as well as mean aflatoxin values for selected maize inbred lines evaluated in different confirmation tests conducted at the Southern Regional Research Center laboratory in New Orleans, LA, using kernel-screening assay.

Line	Pedigree	Kernel color	Kernel texture	Line	Resistant check (MI82)	Susceptible check (P3142)	$LSD_{0.05}$	CV%	Among lines
				Aflatoxin mean (ng g^{-1})†					
TZAR101	1368/GT-MAS-Gk-10-3-1-2-B*4	Yellow	Flint	77	92	3629	4200	164	‡
TZAR102	1368/MI82-23-1-1-4-B*4	White	Flint	383	871	4171	808	76	‡
TZAR103	1368/MI82-23-2-1-3-B*4	White	Flint	156	309	1904	3980	82	‡
TZAR104	(GT-MAS:Gk*2/KU1414SR)-8-1-2-3-B*4	Yellow	Flint	11	179	4136	1524	46	‡
TZAR105	(MP420/4001/Mp420)-2-2-3-3-B*4	Yellow	Flint	206	217	2174	929	211	‡
TZAR106	(MP420/4001/Mp420)-3-1-3-1-B*6	Yellow	Flint	26	217	2174	929	211	‡
				Logarithmic mean [log(y + 1) ng g^{-1}]§					
TZAR101	1368/GT-MAS-Gk-10-3-1-2-B*4	Yellow	Flint	2.8	4.0	7.9	3.1	28	‡
TZAR102	1368/MI82-23-1-1-4-B*4	White	Flint	5.4	5.2	8.3	2.1	30	‡
TZAR103	1368/MI82-23-2-1-3-B*4	White	Flint	3.9	3.8	7.5	2.4	26	‡
TZAR104	(GT-MAS:Gk*2/KU1414SR)-8-1-2-3-B*4	Yellow	Flint	1.6	3.7	8.2	2.4	25	‡
TZAR105	(MP420/4001/Mp420)-2-2-3-3-B*4	Yellow	Flint	3.1	1.7	6.7	3.4	128	‡
TZAR106	(MP420/4001/Mp420)-3-1-3-1-B*6	Yellow	Flint	0.7	1.7	6.7	3.4	128	‡

†Original data was used for analysis.

‡Corresponding mean squares significantly different from zero at $P < 0.0001$ levels.

§Data were transformed using log(y + 1) before analysis.

TZAR103 have white kernels, while the remaining four lines have yellow kernels, with all of them showing flint kernel texture (Table 1).

Since measurement of aflatoxin produced by *A. flavus* in maize is a relatively tedious and expensive procedure, it was too costly to assay aflatoxin production in a large number of single plants from the many segregating populations. Assessment of aflatoxin production was, therefore, deferred until homozygous lines (S_5) were developed through selection for agronomic traits and resistance to diseases during the early stages of inbreeding. At the S_1 to S_4 stages of inbreeding, the lines extracted from each biparental cross or backcross were planted in single-rows at Ibadan, Nigeria (7°26′ N, 3°54′ E, altitude 150 m), under severe natural infection with foliar diseases. 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 diseases, including *Puccinia polysora* rust, *Bipolaris maydis* blight, and *Curvularia lunata* leaf spot.

Line Evaluation and Selection

A total of 52, 65, 47, and 56 S_5 lines derived from biparental crosses or backcrosses was planted each in unreplicated 5-m rows at Ibadan in 2002, 2003, 2004, and 2005, respectively. The lines planted in these nursery rows were not artificially inoculated with *A. flavus*. Seed samples of the S_5 lines harvested in each year were sent to the SRRC USDA-ARS laboratory in New Orleans for aflatoxin analysis. These lines were divided into groups, each consisting of 3 to 12 S_5 lines along with the respective resistant parent, as well as resistant and susceptible inbred checks. Each group was screened for resistance to aflatoxin accumulation in a separate experiment using the laboratory-based kernel-screening assay (KSA) as described by Brown et al. (1995). The test for each line was replicated at least eight times in each experiment. Among the 220 S_5 lines evaluated in the different groups, 93 lines, including those slated for release, had aflatoxin values similar to or significantly lower than their respective U.S. resistant recurrent parent or a tropical inbred parent (Menkir et al., 2006). Seed samples of the S_7 generation of these lines grown in unreplicated 5-m rows at Ibadan were sent to the SRRC USDA-ARS laboratory in New Orleans for resistance-confirmation tests. These lines were again divided into 12 groups, containing 6 to 12 lines each, the respective resistant parent, and resistant and susceptible inbred checks, and were reevaluated for resistance to aflatoxin contamination using KSA. Each line was tested in at least six replications in each confirmation experiment.

Fifty lines chosen from among the 93 promising S_5 lines selected for resistance to aflatoxin contamination using KSA were arranged in a randomized complete block design with two replications and evaluated in a field trial in Nigeria in 2007. The lines were artificially inoculated with *A. flavus* to assess the effectiveness of their resistance to a different strain of *A. flavus*. When the developing grains were at the milk stage, cobs of seven plants were inoculated with a highly toxigenic isolate (La3228) of *A. flavus* using the pinbar method of King and Scott (1982) as modified by Abbas et al. (2006) to minimize the chance for escapes. After physiological maturity, the inoculated cobs were dehusked, and the grains were shelled manually to form bulk samples for each plot. A 20-g sample was drawn from each plot and ground to extract aflatoxin with 100 mL of 70% methanol using a high-speed blender, partitioned in methylene chloride, evaporated to dryness, and the residue redissolved in methylene chloride. We spotted the extracts and aflatoxin standards on

thin-layer chromatography (TLC) plates (silica gel 60, 250 μm) and allowed them to be separated using diethyl ether–methanol–water (96:3:1) solvent mixture. Aflatoxin B_1 and Aflatoxin B_2 were quantified using scanning densitometer, CAMAG TLC Scanner 3 with winCATS 1.4.2 software (Camag AG, Muttenz, Switzerland). The 93 promising S_5 lines selected for resistance-confirmation tests were also evaluated in replicated trials at Ikenne, Saminaka, and Zaria, Nigeria, to assess their agronomic performance and resistance to ear rot and foliar disease.

Characteristics

Resistance to Aflatoxin Production

Among the selected lines evaluated in confirmation tests, nearly two-thirds had aflatoxin levels that were lower than that of the elite tropical adapted parent or the recurrent temperate parent (Menkir et al., 2006). Some of the lines also had either similar or lower aflatoxin levels compared with the resistant U.S. inbred check, MI82. The results of confirmation tests of the six germplasm lines selected for registration are given in Table 1. All the lines had aflatoxin levels that were not significantly different from that of the resistant U.S. inbred check. However, the six germplasm lines had significantly lower aflatoxin levels compared with a susceptible check, P3142 (Table 1). These lines also had significantly lower levels of aflatoxin compared with a susceptible tropical commercial inbred check (9071) when they were inoculated with a different strain of *A. flavus* in the field in Nigeria (Table 2). Although the difference between aflatoxin value of each of the six lines and that of the best commercial inbred check (1368) was not significant, the former had 27 to 95% lower B_1 and 31 to 95% lower B_2 values than the latter in this trial (Table 2).

Field Performance

As shown in Table 3, the selected six germplasm lines had acceptable yield potential, good husk cover, desirable plant and ear aspect scores, and good levels of resistance to ear rot, southern corn leaf blight, and southern corn rust. Testcross mean grain yields of most of the resistant inbred lines recorded in different trials at three locations in 2006 varied from 6232 to 9248 kg ha^{-1} (data not shown), all of which were similar to or higher than that of commercial hybrid checks (6030 to 6915 kg ha^{-1}). These testcrosses also had desirable plant and ear aspect scores and were found to be similar to or better than the commercial hybrid checks in terms of resistance to ear rot, southern corn leaf blight, and southern corn rust. The results indicate that the lines selected for resistance to aflatoxin accumulation also had good agronomic traits for use in maize breeding programs.

Since these inbred lines involve parents of both tropical and temperate origin, they likely contain new combinations of complimentary alleles imparting resistance to aflatoxin accumulation. Such lines can be exploited by maize breeders in the United States as new sources of resistance for developing maize cultivars with higher levels of resistance to *A. flavus* infection and aflatoxin contamination. They can also serve as sources of resistance to foliar diseases, as well as desirable agronomic traits, to expand the genetic base of adapted U.S. maize germplasm and ultimately to accelerate the development of productive new cultivars. The resistant lines with good agronomic traits would also have the potential to be used as parents to accelerate breeding for resistance to aflatoxin contamination in the national programs in west and central Africa.

Table 2. Mean aflatoxin values of maize inbred lines selected for low aflatoxin production using kernel-screening assay, which were evaluated in a replicated trial under artificial inoculation in the field at Ibadan, Nigeria, in 2006.

Lines		Aflatoxin†					
		B1	B2	Total	B1	B2	Total
		Aflatoxin mean (ng g^{-1})			Logarithmic mean (log(*y*+1) ng g^{-1})‡		
TZAR101	1368/GT-MAS-gk-10-3-1-2-B*5	271	36	308	5.6	0.0	5.6
TZAR105	(Mp420/4001/MP420)-2-2-3-3-B-B-B-B	1114	105	1219	7.0	2.7	7.1
TZAR106	(Mp420/4001/MP420)-3-1-3-1-B*5	2218	476	2694	7.7	6.0	7.9
TZAR102	1368/MI82-23-1-1-4-B-B-B-B-B	3060	452	3511	7.5	6.0	7.7
TZAR103	1368/MI82-23-2-1-3-B-B-B-B-B	3733	543	4276	7.9	6.1	8.1
TZAR104	(GT-MAS:gk/*2/KU1414SR)-8-1-2-3-B*7	3914	518	4432	8.2	6.2	8.3
TZI3	1368 (Tropical check)	5330	755	6085	8.6	6.6	8.7
TZI35	KU1414-SR (Tropical check)	7906	1066	8972	8.9	7.0	9.1
TZI18	4001 (Tropical check)	9637	1443	11079	8.8	6.9	9.0
TZI12	9071 (Susceptible check)	13361	2077	15439	9.4	7.5	9.6
Mean		7406	1094	8500	8.7	6.8	8.8
$LSD_{0.05}$		7756	1204	8925	1.2	1.9	1.2
CV (%)		52	55	52	7	8	7
Lines		*	*	*	§	§	§

*Mean squares significantly different at $p < 0.05$.

†Aflatoxin B1 and aflatoxin B2 are structurally related distinct and biologically active secondary metabolites produced by *Aspergillus falvus*. Original data were used for analysis.

‡Data were transformed using log(*y* + 1) before analysis.

§Mean squares significantly different at $p < 0.0001$.

Table 3. Mean grain yield and other agronomic traits of selected resistant maize inbred lines to aflatoxin contamination evaluated at Ikenne, Saminaka, and Zaria, Nigeria, in 2004, 2005, and 2006.

Pedigree	Days to anthesis	Days to silking	Plant height	Ear height	Husk cover	Plant aspect	Ear aspect	Ear rot score	Southern corn blight	Southern corn rust	*Curvularia* leaf spot	Grain yield
			cm		1–5[†]	1–5[‡]	1–5[§]	1–5[¶]	1–5[#]			kg ha^{-1}
Trial 1 (2004)												
(GT-MAS:Gk*2/KU1414SR)-8-1-2-3-B-B-B	63	65	160	75	1.8	2.6	2.9	2.5	2.4	1.8	1.5	2283
(Mp420 × 4001 × MP420)-2-2-3-3-B-B-B	61	63	155	68	1.8	2.3	1.9	1.9	2.5	2.0	2.3	3001
(Mp420 × 4001 × MP420)-3-1-3-1-B-B-B	62	64	127	63	1.9	1.8	1.6	1.9	2.0	1.5	1.5	3745
9071 (check)	61	63	135	63	1.7	2.8	2.9	2.6	2.4	2.0	1.8	1618
Mean	61	63	131	58	2.0	2.9	2.8	2.5	2.8	2.0	1.6	2245
SE	0.9	1.2	6.5	4.4	0.2	0.4	0.3	0.3	0.5	0.3	0.3	455.6
Lines	***	**	***	***	*	***	**	*	ns[††]	ns	ns	***
Line × location	***	***	*	**	ns	***	***	***	***			*
Trial 2 (2005)												
1368 ×GT-MAS-Gk-10-3-1-2-B-B-B	61	62	147	59	2.1	2.3	2.6	2.8	3.3	2.0	2.5	2879
KU1414-SR (check)	67	70	139	63	1.8	2.6	3.2	3.0	3.0	2.0	2.5	946
Mean	65	67	123	53	2.0	3.0	3.0	2.6	3.1	2.0	2.5	1098
SE	0.8	0.9	5.7	3.2	0.1	0.2	0.2	0.3	0.2	0.03	0.03	241.6
Lines	***	***	***	***	ns	ns	***	ns	***	ns	ns	***
Line × location	**	**	*	ns	***	***	**					***
Trial 3 (2006)												
1368 × MI82-23-2-1-3-B-B-B	64	66	129	53	2	2	2	2.0	3.3		2.5	1675
1368 × MI82-23-1-1-4-B-B-B	65	66	142	54	2	2	2	1.3	3.0		2.5	1629
1368 (check)	63	65	137	55	2	3	2	1.7	3.3		2.5	1419
Mean	64	66	137	57	2	2	3	2.1	3.1		2.5	1367
SE	0.9	1.2	5.3	3.2	0.1	0.2	0.2	0.4	0.4		0.1	235.5
Lines	***	***	***	***	ns	**	**	*	***		***	***
Line × location	**	***	ns	ns	*	***	**					ns

*Corresponding mean squares significantly different from zero at $P < 0.05$.

**Corresponding mean squares significantly different from zero at $P < 0.01$.

***Corresponding mean squares significantly different from zero at $P < 0.001$.

[†]1 = very tight husk well extended beyond the ear tip, 5 = exposed ear tip.

[‡]1 = excellent overall phenotypic appeal, 5 = poor overall phenotypic appeal..

[§]1 = clean, uniform, large, and well-filled ears, 5 = rotten, variable, small, and partially filled ears.

[¶]1 = little or no visible ear rot and 5 = extensive visible ear rot.

[#]Disease scores recorded at 26 d after midsilking on a 1–5 scale, where 1 = no visible infection and 5 = severe infection on all leaves.

[††]ns, not significant.

Availability

The International Institute of Tropical Agriculture will multiply and maintain Breeder seed of these germplasm lines. For breeding and research use, small quantities of seed of these germplasm lines can be obtained from the leader of the maize breeding unit at IITA, PMB 5320, Ibadan, Nigeria. Seeds of these germplasm lines will also be maintained in the National Plant Germplasm System, where they will be available for research purposes, including development and commercialization of new materials. Recipients of seed are requested to make appropriate recognition of the original seed source when these germplasm lines contribute to research or the development of new lines, hybrids or synthetics.

Acknowledgments

This research was conducted at the International Institute of Tropical Agriculture and financed by FAS USDA-ARS, USAID, and IITA. The authors express their appreciation to all staff members that participated during planting, data recording, harvesting, and management of the trials at the various locations.

References

Abbas, H.K., R.M. Zablotowicz, and H.A. Bruns. 2006. (Abstract.) Assessing the colonization potential of *Aspergillus flavus* strains on corn under field conditions using a pin bar inoculation technique. Phytopathology 96:S2.

Brown, R.L., Z.Y. Chen, A. Menkir, T.E. Cleveland, K. Cardwell, J. Kling, and D.G. White. 2001. Resistance to aflatoxin accumulation in kernels of maize inbreds selected for ear rot resistance in west and central Africa. J. Food Prot. 64:396–400.

Brown, R.L., T.E. Cleveland, G.A. Payne, C.P. Woloshuk, K.W. Campbell, and D.G. White. 1995. Determination of resistance to aflatoxin production in maize kernels and detection of fungal colonization using an *Aspergillus flavus* transformant expressing *Escherichia coli* β-glucuronidase. Phytopathology 85:983–989.

Brown, R.L., P.J. Cotty, T.E. Cleveland, and N.W. Widstrom. 1993. Living maize embryo influences accumulation of aflatoxin in maize kernels. J. Food Prot. 56:967–971.

Kim, S.K., Y. Efron, F. Khadr, J. Fajemisin, and M.H. Lee. 1987. Registration of 16 maize streak virus resistant tropical maize parental inbred lines. Crop Sci. 27:824–825.

King, S.B., and G.E. Scott. 1982. Field inoculation techniques to evaluate maize for reaction to kernel infection by *Aspergillus flavus*. Phytopathology 72:782–785.

McMillian, W.W., N.W. Widstrom, and D.M. Wilson. 1993. Registration of GT-MAS:gk maize germplasm. Crop Sci. 33:882.

Menkir, A., R.L. Brown, R. Bandyopadhyay, Z.-Y. Chen, and T.E. Cleveland. 2006. A U.S.A.–Africa collaborative strategy for identifying, characterizing, and developing maize germplasm with resistance to aflatoxin contamination. Mycopathologia 162:225–232.

Scott, G.E., and N. Zummo. 1992. Registration of Mp420 germplasm line of maize. Crop Sci. 32:1296.