Sources of Resistance to *Aspergillus flavus* and Aflatoxin Contamination in Groundnut Genotypes in West Africa

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ABSTRACT

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Aflatoxin contamination is an important constraint to groundnut production in West Africa. During the 1989, 1990, and 1991 rainy seasons, we tested 25 lines, including germ plasm, advanced breeding lines, and cultivars, from West Africa, at Sadore, Bengou, and Maradi in Niger, at Kaolack in Senegal, and at Niangoloko in Burkina Faso. Average seed infection varied with site and year from 5 to 37%. Cultivars 55-437, J11, and PI 337394 F were the least infected. Among the ICRISAT advanced breeding lines involving parents resistant to *A. flavus*, ICGV 87084, ICGV 87094, and ICGV 87110 were resistant. The results showed that some breeding lines possessed a good level of resistance to *A. flavus*, reflecting the presence of genes for resistance. *A. flavus* infection was significantly correlated with aflatoxin content, ranging from 1 to 450 ppb. Only one line, VAR 27, showed a high percentage of infection by *A. flavus* but a low level of aflatoxin, suggesting that this line may be resistant to aflatoxin production in West Africa. Among the ICRISAT breeding lines, ICGV 87110 had the lowest level of aflatoxin.

Aflatoxin contamination of groundnut (*Arachis hypogaea* L.) is one of the most important constraints to production in many West African countries. The aflatoxin contamination of groundnut is of significance in relation to public health and to future export trade (16,18,19,21). *Aspergillus flavus* Link:Fr. infection occurs under both pre- and postharvest conditions (4,6,9,17). Preharvest infection by *A. flavus* and consequent aflatoxin contamination are important in the semiarid tropics, especially when end-of-season drought occurs (1,7). Drought stress may increase susceptibility to fungal invasion by decreasing the moisture content of the pod and seed or by greatly lowering the physiological activity of groundnut (1,7,13).

One of the possible means of reducing aflatoxin contamination of groundnut is the use of resistant cultivars. Several sources of resistance have been reported from different parts of the world, especially India, Senegal, and the United States (2,5,7,10,14–20,22). We assembled some resistant lines for testing in combination with some breeding lines from the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, and cultivars from West Africa.

The main objectives of this study were to check the stability of reported resistant lines in West Africa, test West African cultivars for their reaction to *A. flavus* and aflatoxin production, and test some advanced breeding lines for resistance to *A. flavus*.

MATERIALS AND METHODS

During the 1989, 1990, and 1991 rainy seasons, 25 lines, including germ plasm, advanced *A. flavus*-resistant breeding lines, and cultivars from West Africa, were tested at Sadore, Bengou, and Maradi in Niger, at Niangoloko in Burkina Faso, and at Kaolack in Senegal;

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a few lines were replaced in 1990 and 1991. Crop duration varied from 100 to 115 days, depending on the genotypes. Each trial was arranged as a 5 × 5 lattice with three replications. Before planting, fields were prepared by animal-drawn ploughs and by broadcasting 40 kg/ha of P_2O_5. At planting, seeds were treated with Thioral (25% heptachlor and 25% thiram), 3 g/kg of seed. Seeds were hand-planted every 10 cm in four rows 4 m long and 50 cm apart. During the cropping season, fields were weeded one to three times with local hoeing instruments or by hand. No other crop protection measures were taken.

Total annual rainfall was 623 mm in 1989, 399 mm in 1990, and 603 mm in 1991 at Sadore, Niger; 694 mm in 1989, 464 mm in 1990, and 393 mm in 1991 at Kaolack, Senegal; and 922 mm in 1989, 1,333 mm in 1990, and 1,367 mm in 1991 at Niangoloko, Burkina Faso.

Two harvests were performed according to the maturity group of the entries. Plants were hand-harvested from each replication, and pods were removed, brought to the crop work area, and exposed to natural air temperature of 30–35 C. After 3–4 days of drying, 300 pods were hand-shelled, and 100 seeds were tested in the laboratory to determine percentage of infection by *A. flavus*. Our survey showed that in all sites, more than 90% of seeds were infected by the *A. flavus* group; therefore, no distinction was made between *A. flavus* and *A. parasiticus* Speare. Seeds were surface-sterilized by soaking for 3 min in a 0.1% aqueous solution of mercuric chloride, rinsed three times with sterile distilled water, and placed on filter paper in 10-cm-diameter sterile petri dishes at 25 C. To maintain high humidity, 1–2 ml of distilled water was added every day during the first 5 days. After 7 days, the number of seeds contaminated by *A. flavus* and other fungi were counted.

The aflatoxin content of each entry was measured by the enzyme-linked immunosorbent assay (ELISA) technique in a bulk sample from the three replications. We used a test kit (Transia, Lyon, France) based on an enzyme-immunoassay reaction using a monoclonal antibody that specifically recognizes aflatoxin B_1, B_2, G_1, and G_2; we detected only aflatoxin B_1. For each sample, 100 g of seed was ground, and a 20-g subsample was used for extraction in an aqueous methanol solution (80%, v/v). Into this subsample, 60 ml of methanol solution was added. The sample was then homogenized at high speed for 3 min and filtered through a Whatman No. 1 filter. We collected 5 ml of the extract for use in ELISA, following the instructions provided by Transia. To determine aflatoxin concentration from each dilution (1:15, 1:75, and 1:375), we placed 50 µl of the diluted extracts in duplicates into the wells. The optical density was read at a wavelength of 450 nm with the aid of a microtitration plate reader.

Statistical analysis was carried out using rough data and arcsine-transformed values, and analysis of variance was performed on the data.

RESULTS

Seed infection by *A. flavus*. *Niger.* At Bengou, the average seed infection was 6% during both 1989 and 1990. In both years, significant differences ($P \leq 0.001$) between genotypes were found. Seed infection by *A. flavus* varied from 1 to 15% in 1989 and from 0 to 54% in 1990. Lines reported as resistant elsewhere, such as 55-437, J11, and Ah 7223, were among the least infected both years (Table 1). Faizpur, ICGV 87111, VAR 27, and ICGV 87101 were among the lines most susceptible to seed invasion by *A. flavus*.

Results obtained from Maradi were similar to those from Bengou. The average seed infection in 1989 and 1990 was 7 and 5%, respectively. All lines that were among the least infected at Bengou were also resistant at Maradi (Table 1). Of the ICRISAT lines that were bred for resistance to *A. flavus*, ICGV 87095, ICGV 87084, and ICGV 87110 were among the least infected, but some other lines, including ICGV 87101 and ICGV 87108, showed variable levels of seed infection in different locations and years.

At Sadore, the average seed infection in 1989, 1990, and 1991 was 20, 25, and 37%, respectively (Table 2). Seed infection varied from 2 to 64% in 1989, from 3 to 66% in 1990, and from 16 to 67% in 1991. Significant ($P \leq 0.001$) differences among genotypes were found. Among the ICRISAT advanced breeding lines, ICGV 87107, ICGV 87094, and ICGV 87110 were the least infected (Table 2). All known resistant lines, including PI 337394 F, J11, 55-437, U4-47-7, and Ah 7223, were among the least infected. Percentage seed infection was highest in VAR 27, CS 52, JL 24, Faizpur, and ICGV 87108.

Senegal. At Kaolack, the average seed infection in 1990 and 1991 was 8 and 10%, respectively (Table 3). Seed infection varied from 2 to 18% in 1990 and from 2 to 29% in 1991. All lines with low percentages of seed infection in other locations were also resistant in Koalack (Table 3). In 1991, there was significant seed damage by the groundnut borer (*Carreydon serratus*). The number of undamaged seeds available for the *A. flavus* infection test varied from 50 to

Table 1. Percentage of groundnut seed contaminated by *Aspergillus flavus* at Bengou and Maradi, Niger, during the rainy season in 1989 and 1990

		Bengou		Maradi	
No.	Entry	1989	1990	1989	1990
1	55-437	1 (6.8)[a]	2 (7.9)	0 (0.0)	2 (8.8)
8	ICGV 87084	2 (6.9)	4 (11.3)	1 (6.6)	9 (17.3)
9	UF 71513-1	2 (6.9)	2 (8.0)	1 (4.4)	3 (9.3)
2	J11	2 (7.4)	1 (3.6)	1 (4.4)	2 (8.1)
3	ICGV 87107	2 (8.6)	2 (6.7)	4 (11.4)	8 (11.3)
7	U4-47-7	2 (8.4)	0 (0.0)	2 (8.5)	2 (7.9)
4	Ah 7223	3 (9.0)	3 (9.3)	1 (4.4)	4 (11.0)
15	ICGV 87095	3 (9.8)	3 (7.7)	12 (20.2)	2 (7.7)
12	ICGV 87106	3 (10.8)	2 (7.4)	7 (14.9)	4 (10.9)
11	U1-2-1	3 (10.9)	1 (6.1)	8 (16.8)	3 (9.9)
16	ICGV 87112	4 (12.2)	7 (15.2)	1 (5.3)	2 (6.9)
6	ICGV 87110	5 (12.4)	2 (7.9)	5 (12.6)	3 (9.8)
10	ICGV 87109	5 (13.0)	2 (8.9)	16 (23.5)	8 (15.6)
19	TS 32-1	6 (13.5)	4 (11.7)	7 (14.9)	8 (15.5)
24	28-206	6 (14.3)	...	13 (20.8)	6 (14.0)
17	ICGV 87111	6 (14.4)	...	3 (10.1)	...
13	ICGV 87089	7 (14.8)	3 (7.6)	7 (14.9)	2 (7.9)
21	Faizpur	7 (14.8)	...	7 (14.9)	...
14	ICGV 86174	7 (15.2)	...	2 (8.5)	1 (5.2)
22	JL 24	8 (16.7)	2 (5.8)	23 (28.1)	...
23	ICGV 87108	10 (18.2)	5 (13.1)	2 (8.3)	16 (23.6)
5	ICGV 87094	11 (18.8)	3 (9.6)	2 (7.6)	2 (7.9)
18	ICGS 76	14 (20.4)	...	4 (12.1)	7 (14.8)
20	ICGV 87101	14 (22.2)	5 (12.8)	1 (4.4)	...
25	VAR 27	15 (22.4)	6 (13.9)	44 (41.8)	8 (16.8)
30	73-30	...	3 (9.1)	...	...
27	69-101	...	19 (25.4)	...	3 (9.9)
32	73-27	...	54 (47.2)	...	...
26	PI 337394 F	...	1 (3.9)	...	1 (5.2)
31	73-33	...	4 (11.6)	...	...
29	CS 52	...	...	...	10 (18.1)
28	756 A	...	...	...	7 (14.8)
SE		(±1.2)	(±2.2)	(1.8)	(1.7)
Trial mean		6 (13.1)	6 (10.9)	7 (12.8)	5 (12.2)
CV (%)		(16.97)	(34.67)	(23.88)	(23.87)
Lattice efficiency (%)		(103)	(101)	(<100)	(<100)

[a] Means of three replications; figures in parentheses are arcsin-transformed values.

Table 2. Percentage of groundnut seed contaminated by *Aspergillus flavus* at Saldore, Niger, during the rainy season in 1989, 1990, and 1991

No.	Entry	1989	1990	1991
1	55-437	2 (8.6)[a]	3 (9.4)	18 (25.2)
2	J11	5 (13.0)	4 (12.6)	17 (24.1)
3	ICGV 87107	6 (14.1)	17 (24.3)	30 (32.7)
4	Ah 7223	7 (16.0)	8 (15.8)	28 (31.6)
5	ICGV 87094	7 (15.1)	17 (23.9)	40 (39.3)
6	ICGV 87110	7 (15.4)	6 (13.3)	42 (40.0)
7	U4-47-7	8 (15.9)	7 (14.3)	40 (39.3)
8	ICGV 87084	10 (18.5)	16 (23.5)	26 (30.1)
9	UF 71513-1	11 (19.1)	10 (16.9)	16 (23.2)
10	ICGV 87109	11 (19.1)	20 (25.4)	34 (35.7)
11	U1-2-1	11 (19.5)	13 (20.5)	39 (38.6)
12	ICGV 87106	12 (20.1)	12 (20.2)	34 (35.8)
13	ICGV 87089	13 (20.9)	28 (30.7)	22 (27.3)
14	ICGV 86174	13 (21.1)	...	...
15	ICGV 87095	15 (22.6)	37 (36.4)	54 (48.0)
16	ICGV 87112	21 (27.7)	38 (37.3)	30 (30.7)
17	ICGV 87111	24 (29.0)	...	...
18	ICGS 76	26 (30.8)	...	...
19	TS 32-1	26 (31.1)	15 (22.5)	43 (40.4)
20	ICGV 87101	28 (31.3)	14 (21.1)	49 (44.9)
21	Faizpur	28 (32.2)	...	...
22	JL 24	34 (35.8)	44 (41.9)	53 (47.0)
23	ICGV 87108	49 (44.0)	62 (52.8)	39 (38.5)
24	28-206	53 (47.1)	31 (33.2)	...
25	VAR 27	64 (53.3)	54 (47.4)	57 (49.0)
26	PI 337394 F	...	4 (11.3)	20 (26.3)
27	69-101	...	41 (40.1)	45 (41.6)
28	756 A	...	56 (48.9)	...
29	CS 52	...	66 (54.4)	67 (57.6)
30	73-30	...	...	48 (43.3)
31	73-33	...	...	31 (33.4)
SE		(±1.8)	(±5.6)	(±5.1)
Trial mean		20 (24.9)	25 (27.9)	37 (36.9)
CV (%)		(12.04)	(35.00)	(23.72)
Lattice efficiency (%)		(103)	(107)	(115)

[a] Means of three replications; figures in parentheses are arcsin-transformed values.

Table 3. Percentage of groundnut seed contaminated by *Aspergillus flavus* at Kaolack, Senegal, during the rainy season in 1990 and 1991

No.	Entry	1990	1991
15	ICGV 87095	2 (6.0)[a]	5 (12.1)
6	ICGV 87110	2 (6.2)	8 (16.2)
26	PI 337394 F	2 (7.2)	9 (15.5)
24	28-206	3 (9.5)	4 (11.3)
23	ICGV 87108	4 (10.7)	7 (14.2)
7	U4-47-7	5 (12.1)	2 (8.5)
1	55-437	5 (12.5)	7 (14.3)
31	73-33	5 (12.8)	21 (26.9)
8	ICGV 87084	6 (13.6)	15 (22.6)
12	ICGV 87106	6 (13.6)	5 (11.1)
2	J 11	6 (14.1)	5 (10.6)
27	69-101	6 (14.2)	26 (26.9)
11	U1-2-1	6 (14.2)	11 (17.6)
13	ICGV 87089	8 (15.6)	7 (12.2)
16	ICGV 87112	8 (15.6)	5 (12.2)
9	UF 71513-1	8 (15.8)	6 (13.9)
19	TS 32-1	8 (16.3)	9 (16.9)
4	Ah 7223	9 (17.7)	12 (20.6)
5	ICGV 87094	10 (17.8)	4 (10.5)
3	ICGV 87107	10 (18.6)	9 (15.8)
22	JL 24	11 (17.8)	8 (16.1)
10	ICGV 87109	12 (20.2)	7 (14.3)
30	73-30	12 (18.6)	8 (15.2)
20	ICGV 87101	17 (24.0)	13 (25.8)
25	VAR 27	18 (24.2)	29 (32.0)
SE		(±3.4)	(±4.7)
Trial mean		8 (15)	10 (16)
CV (%)		(39.11)	(50.42)
Lattice efficiency (%)		(<100)	(<100)

[a] Means of three replications; figures in parentheses are arcsin-transformed values.

100 for different entries. This contributed to the high variation of results in 1991.

Burkina Faso. Because seed infection by *A. flavus* at Niangoloko was very low in both 1990 and 1991, varying from 0 to 2%, differences between genotypes could not be detected. The low rate of aflatoxin infection in this region of Burkina Faso is mainly due to high rainfall (900–1,300 mm) during the cropping season until harvest. Aflatoxin infection in this part of the country, however, is a serious problem during storage.

Aflatoxin contamination. Aflatoxin contamination was highest at Sadore. Only the results of the 1990 and 1991 trials are presented.

In 1990, aflatoxin contamination varied from 1 to 450 ppb and averaged 143 ppb. Contamination was high in all susceptible lines showing high rates of *A. flavus* infection. Lines with low rates of *A. flavus* infection, including 55-437, J11, PI 337394F, U4-47-7, and Ah 7223, showed low levels of aflatoxin (Fig. 1). The most susceptible lines, including CS 52, JL 24, and 69-101, showed high aflatoxin levels. Only VAR 27, which was highly susceptible to *A. flavus*, showed a very low level of aflatoxin. Among the ICRISAT breeding lines, ICGV 87110 produced the lowest level of aflatoxin (Fig. 1).

Average seed contamination by aflatoxin was higher in 1991 than in 1990. However, some lines, such as J11, 55-437, and PI 337394 F, showed low levels of aflatoxin both years. The genotype CS 52 was most susceptible, with 67% seed invasion by *A. flavus* and 704 ppb of aflatoxin (Fig. 2). Among the ICRISAT breeding lines, ICGV 87110 showed a low level of aflatoxin, although it was higher than in 1990. Line VAR 27 was again the exception to the general trend, showing 49% seed invasion by *A. flavus* but having only 1 ppb of aflatoxin (Fig. 2).

DISCUSSION

Although percentage of seed infection by *A. flavus* in Niger differed according to location and year, a combined analysis showed no genotypes × site interaction. This indicated consistency of genotypes resistant to *A. flavus* across sites.

In all 3 yr, seeds collected from the field at Sadore showed higher infection rates than those at Bengou, Maradi, Kaolack, and Niangoloko. This is mainly due to the low rainfall, sandy soil structure, and insect (chiefly termite) activity at the end of season (8). The rainfall at Sadore was 399 mm in 1990 and 603 mm in 1991, but the month of September (groundnuts are still in the field) was under severe drought stress both years. It is well known that drought at the end of the cropping season facilitates aflatoxin contamination of groundnut (2,13). Genotypes showed substantially different levels of *A. flavus* infection and aflatoxin contamination at Sadore, where conditions are conducive to high disease

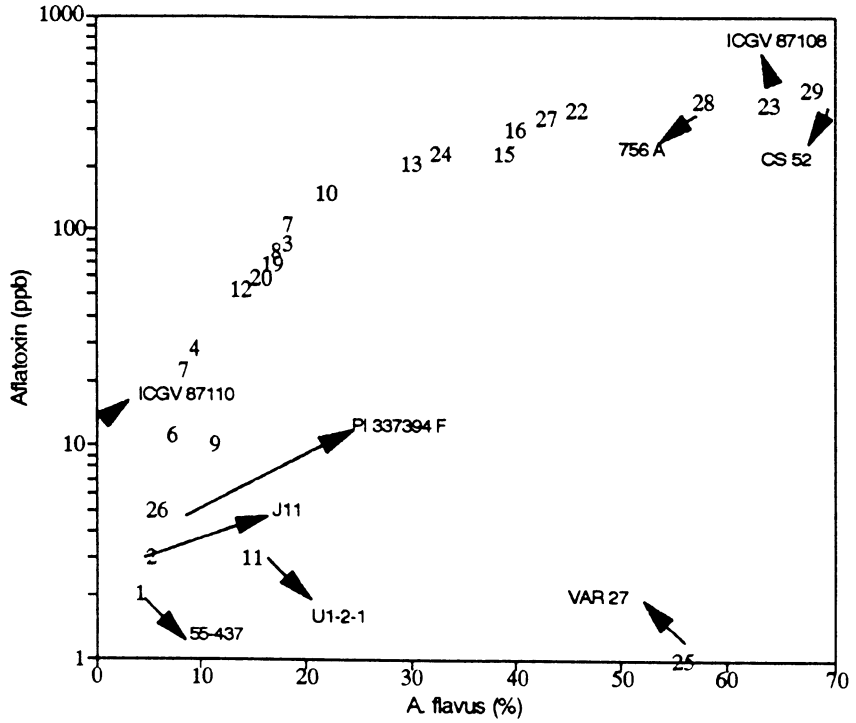

Fig. 1. Percent seed infection by *Aspergillus flavus* and aflatoxin content of groundnut entries tested at Sadore, Niger, during the rainy season in 1990. Entries are identified by numbers (see Table 2).

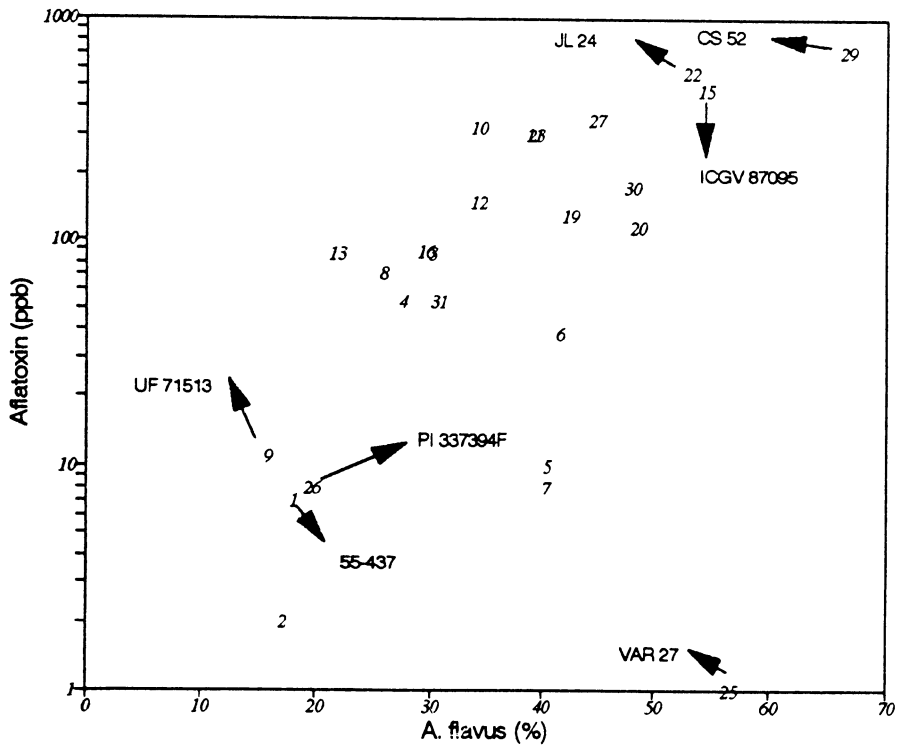

Fig. 2. Percent seed infection by *Aspergillus flavus* and aflatoxin content of groundnut entries tested at Sadore, Niger, during the rainy season in 1991. Entries are identified by numbers (see Table 2).

pressure. Therefore, screening for resistance to *A. flavus* and aflatoxin should be done first in this location; multilocational tests can be done later on selected lines to confirm their resistance.

Germ plasm lines reported resistant to *A. flavus* elsewhere were also found to be resistant in Niger, indicating that resistance is stable across locations. Among these, entry 55-437 from West Africa showed the same level of resistance at all test locations. Several lines showed very high seed colonization by *A. flavus*, and most of these lines also produced high levels of aflatoxin. Only one line, VAR 27, showed a very high level of *A. flavus* with a small quantity of aflatoxin, indicating that this line possesses resistance to aflatoxin production. This type of resistance has already been reported in India (11,21). Such lines could be useful in developing cultivars that combine seed resistance and low capacity for aflatoxin production.

None of the lines reported as resistant possessed a high level of resistance to *A. flavus* at Sadore. However, given the seriousness of aflatoxin contamination to groundnut production in West Africa, it is necessary to exploit these low levels of genetic resistance in an integrated pest management approach. This means that crosses to generate new cultivars in the region should involve resistant parents and that progeny should be screened for resistance at the earliest possible stage. As no high levels of resistance to *A. flavus* have yet been found, even within wild *Arachis* spp. (12), another possible way of developing resistance may be use of molecular techniques to incorporate genes from other genera (3,21), if they exist.

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