Varietal Resistance in Peanut to Aflatoxin Production[1]

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ABSTRACT

Rehydrated, mature, undamaged seed of 502 peanut (*Arachis hypogaea* L.) genotypes were scarified, inoculated with an aflatoxigenic strain of *Aspergillus flavus* Link, and tested for aflatoxin B_1 production after incubation at 25 C for 10 days. All genotypes supported production of aflatoxin B_1 but significant genotypic differences in levels of aflatoxin B_1 production were found. Genotypes U 4-7-5 and VRR 245 supported the lowest levels of aflatoxin B_1 (< 10 μg/g seed), whereas the commonly grown Indian cultivar TMV 2 supported production of aflatoxin B_1 at levels of over 150 μg/g seed. Eight genotypes with low, moderate or high capacity to support aflatoxin B_1 production were further tested using seed from one rainy season crop, and two irrigated postrainy season crops. Genotypic differences in levels of aflatoxin B_1 production were consistent over seasons. Production levels were slightly lower in seed from the rainy season crop than in seed from the two postrainy season crops.

Key Words: Groundnuts, *Aspergillus flavus* Link, *Arachis hypogaea* L., mycotoxins.

Aflatoxin contamination occurs when peanuts (*Arachis hypogaea* L.) are colonized by aflatoxigenic strains of fungi of the *Aspergillus flavus* group, a common occurrence in most countries where the crop is grown (1,4). Levels of contamination can be greatly reduced by prevention of drought stress, timely harvesting, rapid drying of the crop, avoidance of damage to pods and seed by pests, and dry pest-free storage (5), but there are limitations to carrying out these practices, especially in less developed countries. An alternative approach to prevention of aflatoxin contamination is to grow peanut cultivars with resistance to seed invasion by *A. flavus* Link (10, 11, 12, 13). Laboratory inoculation tests have been used to identify several genotypes with resistance to *A. flavus* invasion of rehydrated, undamaged, mature, stored seed (10, 11, 12, 13). However, limited field trials in Georgia, U. S. A., with a few of these genotypes failed to show any reduction in aflatoxin content of their seed compared with that of the commonly grown Florunner cultivar (3, 4).

Another approach to the problem is to search for peanut genotypes that do not support production of aflatoxin when seed are colonized by aflatoxigenic strains of *A. flavus*. Rao and Tulpule (16) tested 60 genotypes and reported that one of them, US 26, did not support aflatoxin production. Kulkarni *et al.* (7) reported that the red-seeded cultivar Asiriya Mwitunde supported only very low levels of aflatoxin production. Although these reports were not confirmed by later research, there were indications of differences between genotypes in ability to support aflatoxin production (2, 6, 14, 17).

In 1979 research was started at ICRISAT to screen the world collection of 11,488 peanut germplasm accessions to identify genotypes that did not support, or poorly supported aflatoxin production. Significant varietal differences in rate and total accumulation of aflatoxin were found between some genotypes (9). This paper presents further data on the comparative abilities of different genotypes to support aflatoxin production following inoculation of rehydrated, sound, mature, stored seed with an aflatoxigenic strain of *A. flavus*.

[1]Submitted as Journal Article No. 548 by the International Crops Research Institute for the Semi-Arid Tropical (ICRISAT).

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Materials and Methods

All crops were grown on alfisols at ICRISAT Center farm (18° N, 78° E), Hyderabad, India. Rainy season crops were sown in late June and harvested in October; postrainy season irrigated crops were sown in late November, and harvested in April. Sixty kg/ha of P_2O_5 was applied at land preparation and 400 kg/ha of gypsum at pegging. Crops were irrigated were necessary to prevent drought stress. Test genotypes were arranged in randomized block designs with two replications.

All genotypes were harvested at maturity. Plants were arranged in windrows with pods exposed and left to dry for 3 days in the rainy season and 2 days in the postrainy season. Full-sized, mature, undamaged pods were picked from the plants and dried under shade to a seed moisture content below 8%. They were then stored in cloth bags at room temperature until required for testing.

Screening Trials - Genotypes were grown in three crop seasons, 219 in the 1980 rainy season, 181 in the 1981 rainy season, and 102 in the 1982/83 postrainy season. Two samples of seed of each genotype from each of the two replicate plots were tested for aflatoxin B_1 production 2-3 months after harvest.

Additional Tests - Eight genotypes from the 1982/83 screening trial were grown again in the 1983 rainy and 1983/84 postrainy season. They were selected on their abilities to support low (U 4-7-5, VRR 245), moderate (Ah 813, Ah 1069, 26-5-1, C No. 56-106), and high (TMV 2, J11) levels of aflatoxin B_1 production. Two samples of seed of each genotype from each of the two replicate plots from all three trials were tested together on three separate dates in 1984 (13/5/84; 28/7/84; 12/9/84) for ability to support aflatoxin B_1 production.

Aflatoxin Production Tests - Pods were hand-shelled and shrivelled or damaged seed were rejected. Undamaged, mature seed were weighed out into 20 g lots, sterilized by soaking for 3 min in a 0.1% aqueous solution of mercuric chloride, rinsed in two changes of sterile distilled water, and placed in sterile beakers. Sufficient sterile distilled water was then added to each lot to raise the seed moisture content to 20%. The seed were then placed in 9 cm diameter petri-dishes, and their testas scarified with sterile needles. They were then surface inoculated with 1 mL of the spore suspension (4×10^6 conidia/mL) of an 8-day-old culture of an aflatoxigenic strain (AF 8-3-2A) of *Aspergillus flavus*. This strain produces only aflatoxin B_1 (8). Plates were incubated at 25 C for 10 days, then seed were checked for colonization by *A. flavus* prior to determination of their aflatoxin B_1 content.

Seed were dried in a forced draft oven at 60 C for 1 hour, ground, and aflatoxin B_1 extracted and estimated using the method described by Pons *et al.* (15).

Results

All 502 genotypes supported aflatoxin B_1 production, but there were significant differences between them in amounts of aflatoxin B_1 produced. The ranges of aflatoxin B_1 production were 33-176 μg/g seed, 32-125 μg/g seed, and 7-195 μg/g seed for the 1980, 1981 and 1982/83 seasons, respectively. Genotypes were classified into nine arbitrarily fixed categories of aflatoxin B_1 production for seed sampled from the 1980 and 1981 rainy season and 1982/83 postrainy season trials (Table 1). Most genotypes supported production of between 26 and 100 μg of aflatoxin B_1 per gram of seed, and the commonly grown Indian cultivar TMV 2 supported production of over 150 μg aflatoxin B_1 per gram of seed. The lowest levels of production of aflatoxin B_1 were in seed of the genotypes U 4-7-5 and VRR 245 (< 10 μg/g seed). Eight genotypes were selected from the 1982/83 postrainy season trial to represent high, moderate, and low supporters of aflatoxin B_1 production. Their identities, botanical variety, countries of origin (where known), and aflatoxin B_1levels supported by their seed when tested two months after harvest in 1983, are given in Table 2.

The data obtained from the three tests on material from the 1982/83 and 1983/84 postrainy and 1983 rainy seasons were pooled and analysis of variance was carried out on the pooled, data separately for each of the three groups of genotypes that supported low, moderate, and high levels of production of aflatoxin B_1 since standard errors between seasons for the three different groups were homogenous.

The levels of aflatoxin B_1 production recorded in seed of the eight genotypes from the three seasons in the 1984 tests were about the same order as those recorded on seed from the 1982/83 season tested in 1983 (Tables 2 and 3). Highly significant differences in aflatoxin B_1 levels were found between the different groups of genotypes in the 1984 tests. Levels of aflatoxin B_1 production were slightly lower for all genotypes in seeds from the rainy season (1983) than in seeds from the postrainy seasons (1982/83

Table 1. Distribution of 502 peanut genotypes in nine aflatoxin production categories.

Seasons[b]	Numbers of genotypes in the aflatoxin B_1 production categories								
	1-10[a]	11-25	26-50	51-75	76-100	101-125	126-150	151-175	176-200
Rainy									
1980	0	0	21	86	78	33	0	0	1
1981	0	0	46	79	41	15	0	0	0
Postrainy									
1982/83	4	5	73	18	1	0	0	0	1
Totals	4	5	140	183	120	48	0	0	2

[a] aflatoxin B_1 produced in ug/g seed

[b] Seed from 1980 and 1981 rainy and 1982/83 postrainy seasons at ICRISAT, tested two months after respective harvests.

Table 2. Aflatoxin B_1 production in seed of eight peanut genotypes from the 1982/83 postrainy season at ICRISAT, tested in 1983.

Genotype				Aflatoxin B_1
ICG No.[a]	Identity	Botanical variety	Country of origin	production (μg/g seed)
				High production
221	TMV 2	vulgaris	India	195.0[b]
1326	J 11	vulgaris	India	136.0
				Moderate production
1193	Ah 813	vulgaris	?	85.6
2180	Ah 1069	vulgaris	?	75.0
3560	26-5-1	vulgaris	India	52.2
3588	C No. 56-106	fastigiata	?	28.2
				Low production
4681	U 4-7-5	vulgaris	USA	7.6
7101	VRR 245	vulgaris	India	7.3

[a] ICRISAT groundnut accession number

[b] mean of 2 replicate samples

and 1983/84). However, the aflatoxin B_1 production levels were consistent across seasons and times of testing for genotypes within the groups supporting low and high levels of production. There was more variation within the group of genotypes supporting moderate levels of aflatoxin B_1 production, and significant interactions occurred ($p = 0.01$) between seasons and genotypes, this being most obvious for genotype C No. 56-106 (Table 3).

Table 3. Aflatoxin B_1 production in seed of eight peanut genotypes from the 1982/83 and 1983/84 postrainy and 1983 rainy seasons at ICRISAT, tested in 1984.

Genotype	Aflatoxin B_1 production (µg/g seed)[a]		
	Postrainy seasons		Rainy season
	1982/83	1983/84	1983
High production			
TMV 2	185.9	137.9	117.8
J 11	148.4	100.1	89.9
SE[b]		±3.61	
CV(%)		6.80	
Moderate production			
Ah 813	97.9	62.4	24.8
Ah 1069	66.2	44.8	16.3
26-5-1	55.7	35.6	30.8
C No. 56-106	36.1	26.0	7.5
SE[b]		±1.56	
CV(%)		9.10	
Low production			
U 4-7-5	5.9	5.8	5.2
VRR 245	6.1	5.9	4.4
SE[b]		±0.23	
CV(%)		10.40	

[a]Mean of three tests (13/5/84; 28/7/84; 12/9/84, each on two replicate samples

[b]SE associated with means of genotypes x seasons.

Discussion

The consistent low production of aflatoxin B_1 in seed of peanut genotypes U 4-7-5 and VRR 245 and high production in seed of TMV 2 and J11 support the earlier finding of significant differences in peanut genotypes in their abilities to support aflatoxin production (9). Tulpule *et al.* (17) reported seed of the genotype J11 to be highly resistant to aflatoxin production, but when they later tested seed of this genotype collected from eight different agroecological regions of India, only one sample showed resistance. This variation could be due to lack of uniformity in the seed used for sowing the J11 crops at the eight locations, or to climatic and edaphic factors affecting the chemical composition of the seed in different ways in different locations. It is interesting that in the present studies J11 supported higher than average levels of aflatoxin production. It will be necessary to test seed of genotypes U 4-7-5 and VRR 245 from crops grown in different locations and in different soil types to see if their capacities to support aflatoxin production are affected by environmental factors.

The lower production of aflatoxin B_1 in seed from the rainy season as opposed to postrainy season crops at ICRISAT indicates possible effects of crop environment on subsequent ability of seeds to support production of the toxin (Table 3). Rainy season crops are largely rainfed whereas postrainy season crops are irrigated. Irrigation water at ICRISAT has a pH of 7.5-8.0 and contains appreciable quantities of salts (E.C. = 0.15-0.20 mmho/cm). Temperatures vary considerably between seasons and pod zone soil temperatures for rainy and postrainy seasons have been found to range from 22-26 C and 26-35 C respectively. These factors could well influence the development and composition of seed. Comparisons of the chemical constitutions of seed of different genotypes from different seasons and different soil types may indicate possible mechanisms of resistance to aflatoxin production.

A single strain of *A. flavus*, AF 8-3-2A, was used in the aflatoxin production tests at ICRISAT. This strain produces only aflatoxin B1 and has given consistently high yields of the toxin in many tests. Genotypes U 4-7-5 and VRR 245 will have to be tested for aflatoxin production when their seed are colonized by other aflatoxigenic strains of *A. flavus*, although significant strain by genotype interactions are not expected since Nagarajan and Bhat (14) found that the relative differences in aflatoxin B_1 production by several strains of *A. flavus* were maintained across peanut genotypes.

The low toxin producing genotypes identified do not possess any useful resistance to seed invasion and colonization by *A. flavus*. In laboratory tests on seed from the 1982 rainy season crop, levels of seed colonization by *A. flavus* were 41% for genotypes U 4-7-5 and VRR 245, 35% for TMV 2, and 12% for J11 (Mehan, unpublished data). As previously found (10), there was no apparent linkage between levels of aflatoxin B_1 supported by a genotype and the resistance of rehydrated, stored, sound seed to invasion and colonization by *A. flavus*.

We have tested only a small proportion of the World Peanut Germplasm Collection at ICRISAT and it is possible that in further screening genotypes may be found with even greater resistance to aflatoxin production than those already identified, and perhaps some which combine this with resistance to seed invasion by *A. flavus*. Use of cultivars with field resistance to seed invasion by *A. flavus* and resistance to aflatoxin production together with the currently recommended cultural, postharvest drying, and storage methods could substantially solve the peanut aflatoxin contamination problem.

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Accepted December 23, 1985