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Research on the aflatoxin problem in groundnut at ICRISAT*

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Summary Aflatoxin contamination of groundnut is a serious problem in most groundnut producing countries and as such is given high research priority by the Groundnut Improvement Program of ICRISAT. Since 1979 we have concentrated on selecting cultivars resistant to seed invasion and colonization by toxigenic *Aspergillus flavus*, and/or to aflatoxin production following invasion by the fungus. Resistance to invasion and colonization by *A. flavus* of rehydrated, mature seed has been found, and confirmed, in some cultivars. We have also screened several groundnut cultivars for seed resistance in the field, both under natural conditions and with the inoculum of the fungus added to the soil in the pod zone. Some cultivars with resistance to seed colonization also showed resistance to seed invasion by *A. flavus*. None of the cultivars tested has shown complete resistance to aflatoxin production but significant cultivar differences occurred in the amounts of aflatoxin produced in seeds inoculated with a toxigenic strain of *A. flavus*.

Introduction

Invasion of seed by toxigenic strains of *A. flavus* and consequent aflatoxin contamination is a continuing serious problem in most countries where groundnuts are grown[1]. Farmers of the semi-arid tropics have apparently failed to adopt agronomic measures to minimize aflatoxin contamination. We have therefore concentrated on the utilization of genetic resistance of seed to invasion by the toxigenic *A. flavus* and production of aflatoxin[9,11,12]. Since 1979, a research program has been pursued at ICRISAT to select cultivars resistant to seed invasion and colonization by toxigenic *A. flavus* and to aflatoxin production. This paper reports on screening of germplasm for (a) resistance to *in vitro* seed colonization by *A. flavus* (b) resistance to invasion of seeds by *A. flavus* in the field, both under natural conditions and with inoculum of the fungus added to the soil in the pod zone, and (c) resistance to aflatoxin production following invasion and colonization by a toxigenic strain of the fungus.

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Materials and methods

Source of seed

Seeds of all cultivars/lines tested were obtained from rainy and subsequently post-rainy seasons crops grown in alfisols at ICRISAT Center farm. Cultivars/lines were harvested at maturity and plants arranged in inverted windrows in the field. After windrow drying for 2 days in the post-rainy and 3 days in the rainy seasons the pods were hand-picked and sun-dried on mats until the seed moisture content was below 8 per cent. Dried pods were then stored in cloth bags at room temperature until required for testing.

Seed colonization test

For all trials, undamaged, mature seeds from the stored pods were tested for resistance to colonization by *A. flavus* using a modification[7] of the method described by Mixon and Rogers[12]. For each trial, one lot of 20 g of seed was tested from each plot of each cultivar/line. Seeds were surface-sterilized by soaking them in a 0.1% solution of mercuric chloride for 2 min followed by 2 rinses in sterile distilled water and then their moisture contents were raised to 20%. The rehydrated seeds were placed in sterile Petri plates and surface-inoculated with 1 ml of a conidial suspension (4×10^6 conidia/ml) from 8-day-old cultures of the toxigenic *A. flavus* strain AF 8-3-2A. The percentage of seeds colonized was recorded after 8 days of incubation at 25°C.

Effect of soil inoculation on seed infection by A. flavus

Replicated field trials were carried out in the 1979/80 and 1980/81 post-rainy and 1980 and 1981 rainy seasons in which some cultivars/lines were examined for the effects on seed infection of applying inoculum of the toxigenic *A. flavus* strain AF 8-3-2A to the soil around pods at 30 days before harvest. The trials were laid out in a 3-replicated split-plot arrangement, the main plots assigned to inoculation *versus* control and the subplots to test cultivar reaction. Ten randomly selected plants in each replicated plot of each cultivar/line received 500 ml inoculum (15×10^6 conidia/ml) of *A. flavus*. After completion of postharvest drying, replicate samples of 100 undamaged, mature seed were surface sterilized and plated out on Czapek Dox Rose Bengal Streptomycin agar for isolation of *A. flavus*. The plates were incubated at 25°C and colonies of *A. flavus* growing from infected seeds were recorded after 5–7 days.

Aflatoxin production test

Aflatoxin production tests were carried out on seeds of each cultivar/line by the method described by Mehan and McDonald[7]. The rehydrated seeds in replicate plates had their testas damaged by scraping them with a sterile needle. The seeds were then surface-inoculated with the toxigenic strain of *A. flavus* as described above for the seed colonization test. After 10 days of incubation aflatoxins were extracted using the method of Pons' *et al.*[15] and quantitative determination made by the method of Nabney and Nesbitt[14].

Results and discussion

Of 850 cultivars/lines screened, eight showed resistance to seed colonization by *A. flavus*. Three breeding lines (PI337394F, PI337409 and UF71513), reported resistant in the U.S.A.[12,3] were also resistant in the ICRISAT tests, and five new sources of resistance were identified in 1981[8]. These cultivars/lines were further tested in the 1981/82 postrainy and 1982 rainy seasons and the seed resistance was confirmed (Table 1). Although percentages of seeds colonized by *A. flavus* were

Table 1. Groundnut seed resistance to colonization by *Aspergillus flavus* in tests at ICRISAT Center, 1981/82

Cultivar/line	Percentage of seeds colonized	
	Postrainy season 1981/82	Rainy season 1982
PI 337394F	18.0	10.3
PI 337409	18.1	9.1
UF 71513	15.3	8.8
J11	17.7	10.4
Ah 7223	15.8	8.6
Var. 27	19.4	12.4
Faizpur	18.1	12.1
C55-437	*	17.3
TMV_2[a]	46.9	34.4
OG 43-4-1[b]	99.2	94.7
SE ±	1.20	1.11
CV (%)	7.19	8.84

[a] Susceptible check
[b] Highly susceptible check
* Not tested

low in both seasons for resistant cultivars/lines, levels were generally lower in seeds from the rainy season crop than from the postrainy season crop. This finding agrees with those of previously unreported trials carried out in the 1980/81 and 1981 seasons which showed that seed colonization of cultivars/lines by *A. flavus* could be significantly influenced by season, crop location (field) and rate of postharvest drying[10]. High temperatures during postharvest drying of the irrigated postrainy season crop could damage the seed testa and contribute to increased levels of seed colonization[8]. In all reported cases of mature dried groundnut seed resisting colonization by *A. flavus* the protective role of the seed testa has been emphasized[4,13]. The maximum advantage that can be derived from groundnut cultivars resistant to *A. flavus* will only occur if the seed testa is not damaged during cultivations, harvesting, curing, decortication or storage. Resistance in these cultivars/lines to seed invasion and colonization by *A. flavus* is likely to be of value in the event of stored groundnuts absorbing sufficient moisture to permit fungal growth. Also, it would perhaps be useful in seasons when field drying conditions were unfavourable for example, because of late rains. There is some experimental evidence[17] that the cultivars with seed resistance to colonization may have some pre-harvest field resistance to seed invasion by the fungus.

Experiments were carried out to investigate possible varietal differences with regard to pre-harvest resistance to seed infection with *A. flavus* in the field. The results of effects on seed infection of

Table 2. Infection with *Aspergillus flavus* of seeds from field dried pods of cultivars/lines following inoculation with the fungus of soil around developing pods 30 days before harvest

	Percentages of seeds infected with *A. flavus*							
	Seed from:							
	Postrainy season crops				Rainy season crops			
	1979/80		1980/81		1980		1981	
Cultivar/line	Inoc.	No.Inoc.	Inoc.	No.Inoc.	Inoc.	No.Inoc.	Inoc.	No.Inoc.
PI 337394 F	1.6	0.3	2.6	1.0	1.0	0.3	2.3	0.6
PI 337409	3.0	0.6	4.0	1.6	1.6	0.3	2.6	0.6
UF 71513	2.0	0.6	2.6	1.3	1.0	0.0	2.0	0.3
J 11	1.3	0.3	2.6	0.3	1.3	0.6	1.6	0.3
TMV 2	6.6	1.3	7.3	2.6	5.6	1.6	4.6	1.3
Krapovicka Strain #16	4.0	1.6	6.0	2.3	4.6	2.0	*	*
EC 76446 (292)	6.0	1.3	6.6	3.3	6.3	2.0	7.3	1.6
OG 43-4-1	9.0	1.6	7.3	2.3	5.0	1.3	*	*
Robut 33-1	8.0	1.3	11.3	2.3	7.0	1.3	*	*
M 13	6.3	2.0	8.6	3.0	7.3	2.0	*	*
SE ±								
For Inoculation Treatments		0.82		0.97		0.64		0.56
For cultivars		0.68		0.90		0.59		0.55
C.V. (%)								
For Inoculation Treatments		39.27		31.84		27.05		26.02
For cultivars		28.28		27.88		31.41		25.72

* Not Tested; Inoc. = Soil Inoculation; No. Inoc. = No Soil Inoculation

applying inoculum of a toxigenic strain of *A. flavus* to the soil around developing pods of cultivars resistant and cultivars susceptible to seed colonization by the fungus are given in Table 2. The inoculation of soil around developing pods significantly increased seed infection with *A. flavus*. Cultivars/lines differed significantly for levels of *A. flavus* infection and the cultivar times inoculation treatment interaction was significant. Natural *A. flavus* seed infection was also lower in cultivars/lines whose rehydrated stored seeds were resistant to colonization by the fungus than in susceptible cultivars. Further experiments on field inoculation techniques to evaluate cultivars for reaction to seed infection with *A. flavus* and to formation of aflatoxins are in progress.

Previous research indicated varietal differences in aflatoxin production when autoclaved seeds were colonized by toxigenic strains of *A. flavus* and *A. parasiticus*[6, 16]. Although claims of resistance to aflatoxin production were not confirmed by later research[2, 5], quantitative varietal differences in aflatoxin production were indicated. Of 195 cultivars tested at ICRISAT none has proved to be totally resistant to aflatoxin production. Some test data are presented in

Table 3. Aflatoxin B_1 production in groundnut cultivars/lines following inoculation with *A. flavus* strain AF8-3-2A – 1981 rainy season

Cultivar/line	Aflatoxin B_1 (μg/g seed)
PI 337394 F	115.1
PI 337409	104.5
UF 71513	110.7
J11	122.6
Ah 7223	117.4
Var. 27	97.8
Faizpur	118.8
Monir 240-30	103.8
TMV_2	241.5
FESR-11-P11-B2-B1	52.6
OG 43-4-1	70.4
SE ±	2.83
CV (%)	4.29

Table 3. Aflatoxin was produced in all cultivars tested, but cultivar differences in amounts of the toxin produced were found. Some of the cultivars with good resistance to colonization by the fungus proved good substrates for aflatoxin production, while some others that were highly susceptible to fungal colonization were not as good substrates for the production of the toxin. There was no correlation between resistance to seed colonization by *A. flavus* and the ability of seed to support aflatoxin production. The cultivar FESR-11-P11-B2-B1 which was highly susceptible to seed colonization by *A. flavus*, had the lowest level of toxin produced. We have tested only a small proportion of the world groundnut germplasm collection and it is hoped that we may find cultivars with even higher levels of resistance to aflatoxin production and perhaps cultivars which combine good resistance to aflatoxin production with good resistance to seed invasion by the fungus.

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