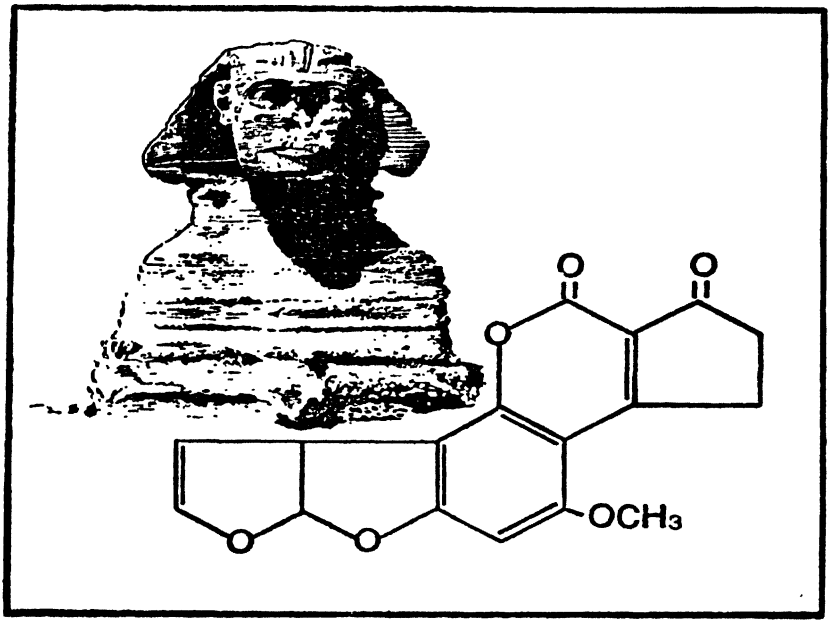
PROCEEDINGS OF THE INTERNATIONAL SYMPOSIUM ON MYCOTOXINS



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Aflatoxin Production in Groundnut Cultivars Resistant and Susceptible to Seed Invasion by Aspergillus flavus

V.K. MEHAN and D. McDONALD

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Groundnut Improvement Program, Patancheru P·O·502 324 , A·P., India

Abstract

The use of groundnut cultivars resistant to seed invasion and colonization by Aspergillus flavus is a possible means of preventing or reducing aflatoxin contamination. Such resistance was identified in several cultivars, one of which was the released commercial Indian cultivar JH. No direct relationship was found between resistance to seed colonizaion by A. flavus and the quantity of aflatoxins produced when seeds were colonized by toxigenic strains of the fungus. Some of the cultivars with seed resistance to A. flavus colonization have been found resistant to fungal pod rot.

Introduction

Contamination of groundnuts with aflatoxin is a serious problem in many parts of the world. The use of groundnut cultivars resistant to seed invasion and colonization by toxigenic strains of *Aspergillus flavus* has been suggested as an effective means of preventing or at least reducing aflatoxin contamination (1-3). Early research reported varietal differences in aflatoxin production when autoclaved seeds were colonized by toxigenic strains of *A. flavus* (4,5). Although claims of resistance to aflatoxin production were not confirmed by later research (6,7), quantitative varietal differences in aflatoxin production were indicated.

This paper reports on screening of germplasm for resistance to seed invasion and colonization by *A. flavus* and for resistance to aflatoxin production when seeds are colonized by toxigenic strains of the fungus.

Submitted as Conference Paper No. CP-49 by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru P O 502 324, A.P., India

Materials and Methods

Source of Seed

Seeds of all cultivars were obtained from the 1979 and 1980 rainy season and the 1979 -80 and 1980-81 post-rainy season crops grown at ICRISAT Center, Patancheru, A.P., India. The crops were not subjected to drought stress. Cultivars were harvested at optimum maturity and plants were arranged in windrows in the field with pods exposed. 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 seed moisture contents were reduced to less than 7%. Pods were then stored in cloth bags in the laboratory until seeds were required for testing.

Seed Colonization Test

The Mixon and Rogers method (1) with some modification (8) was used. For each cultivar, 3 plates of surface-sterilized (soaked in 0.1% aqueous solution of mercuric chloride for 2 min followed by 4 rinses in sterile distilled water) hydrated seeds were inoculated with a spore suspension (4.0×10^6 condia /ml) of the toxigenic *A. flavus* strain AF8-3-2A. The plates were incubated at 25° C and percentages of seeds colonized were recorded after 8 days.

Aflatoxin Production Test

Using seed from the 1980 rainy season crop, aflatoxin production tests were carried out on each cultivar by the method described by Mehan and McDonald (8). Surface-sterilized seeds in 3 plates had their testas damaged by scraping them with a sterile needle. The seeds were then inoculated with a spore suspension of *A. flavus* strain AF8-3-2A and incubated as described above for the seed colonization test. After 10 days of incubation, aflatoxins were extracted using Romer's method (9), determined quantitatively by the method of Nabney and Nesbitt (10), and expressed as micrograms per gram of seed.

Results and Discussion

From 150 cultivars screened, 8 showed resistance to seed colonization. Mean seed colonization percentages are shown in Table 1 for the 8 resistant cultivars, and 2 control cultivars, the susceptible TMV 2 and the highly susceptible OG43-4-1, for seeds from 2 rainy season and 2 post-rainy season crops. The cultivars Ah 7223, Monir 240-30, and UF 71513 showed the highest resistance. Less resistant, but still significantly more resistant than TMV 2, were cultivars J 11, Faizpur, Var. 27, PI 337394 F, and PI 337409. PI 337394 F and PI 337409 have been reported resistant in the United States and in Senegal (2). These 2 cultivars are not agronomically acceptable but J 11 is a released commercial cultivar in India. This cultivar and the other *A. flavus*resistant cultivars Ah 7223, Monir 240-30, and Var. 27 have also been found resistant to a fungal pod rot at ICRISAT Center.

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It is evident that levels of seed colonization on all cultivars were higher on seed from post-rainy season crops than on seed from rainy season crops. Fluctuation in soil moisture during pod development and high temperatures during post-harvest drying of the irrigated post-rainy season crop have been suggested as possible reasons for this difference as these factors could cause damage to the seed testas. This is being investigated.

Early reports of resistance to aflatoxin production (4,5,12) were not substantiated but indications were obtained of possible genetic differences in amounts of aflatoxin produced (6,7,13). Table 2 gives aflatoxin B_1 production figures for 7 of the *A. flavus*-resistant cultivars, for the susceptible TMV 2, and for the highly susceptivle OG-43-4-1 and FESR-11-PII-B2-B1. All cultivars

TABLE 1. Percent seed colonization by *A. flavus* of selected resistant and susceptible cultivars

Cultivar	Source	Percent seed colonized			
		Rainy sensons		Post-rainy sensons	
		1979	1980	1979/80	1980/8[illegible]
UF 71513	USA	—	6 6	13 1	14 2
PI 337394 F . .	Argentina	7 2	8 1	19 9	19 5
PI 337409 . .	Argentina	8 8	9 4	20.8	21 0
J 11	India	9.8	12 0	21.8	17.5
Ah 7223	Nigeria	—	4 5	15 7	15 8
Monir 240-30 . .	-	—	9.0	16 1	14 3
Var.27	Australia	—	9 7	21 2	19 9
Faizpur	India	—	9 8	19 7	19 1
TWV 2[d]	India	34 8	36 7	49 4	41 5
OG 43-4-1[c] . . .	India	94 9	91 2	96 9	98 2
CD (at 5%) . .		3 4	4 0	3 2	4 2
CV (%)		5 9	11 9	5 4	8 8

a Mean of 3 replications. — = not tested

b Susceptible check CV. *c* highly susceptible CV

TABLE 2. Aflatoxin B_1 production in groundnut cultivars resistant and susceptible to seed invasion by toxigenic *A. flavus*

Cultivar	Reaction to seed invasion by *A. flavus*	Aflatoxin B_1 (μg/g seed)
PI 337394 F	Resistant	106.4
PI 337409	Resistant	95.5
J 11	Resistant	117.8
Ah 7223	Resistant	115.2
Monir 240-30	Resistant	93.6
Var.27	Resistant	90.3
Faizpur	Resistant	113.5
TMV 2	Susceptible	226.2
FESR-11-P11-B2-B1	Highly susceptible	50.0
OG 43-4-1	Highly susceptible	76.3
SD (at 5%)		13.2
CV (%)		7.1

supported production of aflatoxin B_1 and there were significant differences between them in the amount produced. There was no correlation between resistance to seed invasion and colonization by *A. flavus* and the ability of the seed to support aflatoxin production. The cultivar FESR-11-P11-B2-B1, which is known to be highly susceptible to seed invasion and colonization by *A. flavus* (13), had the lowest level of toxin production (Table 2). In another experiment, the rate of accumulation of aflatoxin B_1 was shown to be slower in this cultivar than in cultivars TMV 2, J 11, and PI 337409 (Fig. 1).

It would be most useful if cultivars could be developed which combined seed resistance to invasion and colonization by *A. flavus* with resistance to aflatoxin production in the event of the seeds being colonized by a toxigenic strain of the fungus.

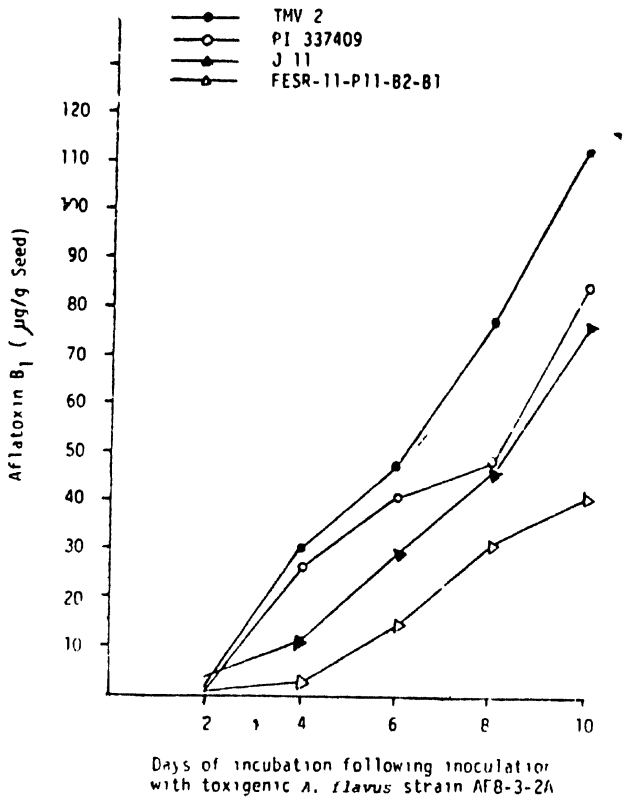

Fig. 1. Aflatoxin B_1 accumulation in inoculated seeds of 4 cultivars incubated at 25 ± 3°C.

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Epidemiology and control of groundnut bud necrosis and other diseases of legume crops in India caused by tomato spotted wilt virus

D.V.R. REDDY, P.W. AMIN, D. McDONALD & A.M. GHANEKAR
Groundnut Improvement Program, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru P.O., A.P. 502 324, India

INTRODUCTION

Tomato spotted wilt virus (TSWV) was first reported in India in tomato in 1964 (Todd *et al.*, 1975). The occurrence of TSWV on a legume in India was first recorded in 1968 (Reddy *et al.*, 1968). The "bud necrosis disease" of groundnut, caused by TSWV, is now considered to be one of the most damaging groundnut diseases in India (Ghanekar *et al.*, 1979a; Reddy, 1980). Bud necrosis is likely to have been present in India for some time although it has only recently become economically important. TSWV has also been reported on groundnuts in Brazil (Costa, 1941), the United States of America (Halliwell & Philley, 1974), South Africa (Klesser, 1966) and Australia (Helms *et al.*, 1961). This chapter considers the epidemiology and control of bud necrosis and gives a brief account of other economically important diseases of legumes in India caused by TSWV.

OCCURRENCE AND DISTRIBUTION OF LEGUME DISEASES CAUSED BY TSWV IN INDIA

Our surveys show that bud necrosis is widely distributed in the main groundnut-growing regions of India and that it is endemic in the states of Andhra Pradesh and Tamilnadu. Extensive infection has also been seen in parts of the states of Maharashtra, Gujarat, Rajasthan and western Uttar Pradesh. The greater incidence of bud necrosis in recent years may be related to the expansion of irrigation projects which has led to continuous cropping of groundnuts and other hosts of TSWV. Until recently most groundnuts were grown in the rainy season but increased demand has caused an expansion of the post-rainy season, irrigated crop.

The economically important leaf curl diseases of green and black gram (*Vigna radiata*) (Nene, 1972) have recently been shown to be caused by TSWV (Ghanekar *et al.*, 1979b) and field trials showed that TSWV can also cause economically important diseases of pea (cultivar Bonneville), broad bean (cultivar Local), cowpea (cultivar C-152) and soyabean (cultivar Bragg).

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