

***Aspergillus flavus* Resistance Breeding in Groundnut: Progress made at ICRISAT Center**

**M.J. Vasudeva Rao, S.N. Nigam, V.K. Mehan,
and D.McDonald¹**

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Abstract

*Progress worldwide in breeding groundnuts resistant to seed colonization by *Aspergillus flavus* and aflatoxin contamination is summarized, and research at ICRISAT described. Resistance to *A. flavus* infection may occur at various levels, but efforts to breed for resistance have concentrated on the utilization of the resistance in the testae of mature seeds. At the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), genotypes identified as resistant to in vitro seed colonization by *A. flavus* have been crossed with susceptible cultivars of good agronomic character, and several breeding lines with stable resistance to seed colonization and with acceptable yield and quality have been produced. The genetics of inheritance of testa resistance is discussed. It is important that when breeding for resistance to *A. flavus* and aflatoxin production, breeders incorporate other resistance traits.*

Résumé

*Sélection pour la résistance à *Aspergillus flavus* chez l'arachide—résultats obtenus au Centre ICRISAT : Les acquis au niveau international dans la sélection d'arachides résistantes à la colonisation des graines par *Aspergillus flavus* et à la contamination par les aflatoxines sont rappelés. La recherche menée à l'ICRISAT est décrite. La résistance se produit à divers niveaux, mais les travaux de sélection sont axés sur la résistance des téguments des graines mûres. A l'ICRISAT, les génotypes identifiés comme résistants à la colonisation in vitro des graines par *Aspergillus flavus* ont été croisés avec des cultivars sensibles ayant de bonnes caractéristiques agronomiques; ainsi, plusieurs lignées à résistance stable, à bon rendement et de bonne qualité ont été créées. L'hérédité de la résistance du tégument est étudiée. Les sélectionneurs devraient incorporer d'autres caractères de résistance.*

Resúmene

*La selección para lograr resistencia a *Aspergillus flavus* en el cacahuete: Avances logrados en el centro ICRISAT : Los avances logrados a nivel internacional en la selección de cacahuete resistente a la colonización de sus semillas por *Aspergillus flavus* y a la contaminación con*

1. Groundnut Breeder, Principal Groundnut Breeder, Groundnut Pathologist, and Principal Groundnut Pathologist, Legumes Program, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh 502 324, India.

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aflatoxinas, se presentan en forma resumida, y se describen las investigaciones realizadas en el ICRISAT. La resistencia a A. flavus puede presentarse a diferentes niveles, pero los trabajos para lograr la resistencia se han concentrado en la utilización de la resistencia de las testas de las semillas maduras. En el Instituto Internacional de Investigación sobre Cultivos en los Trópicos Semiáridos (ICRISAT), los genotipos identificados como resistentes a la colonización in vitro de los granos por Aspergillus flavus, se han cruzado con cultivares susceptibles con buenas características agronómicas, y se han obtenido varias líneas genéticas de cacahuete con resistencia estable a la colonización de la semilla, y con rendimiento y calidad de semilla aceptables. Se examina la genética de la herencia de la resistencia en las testas. Es importante que cuando realicen la selección para lograr resistencia a A. flavus, los fitomejoradores incorporen simultáneamente otras características de resistencia.

Introduction

Of the several control strategies for *Aspergillus flavus* Link ex Fries in groundnut, breeding for resistance is credited to be a sound, long-term approach to aflatoxin prevention in groundnut (Sanders 1983, Cole 1981, Diener et al. 1982, Mixon 1981, Mehan and McDonald 1984). In this paper, we review the general progress made in this area and describe in detail the progress made at ICRISAT Center. The problems and prospects for developing commercially acceptable cultivars of groundnut with resistance to *A. flavus*, are discussed and future research priorities are considered.

Resistance Traits and Their Possible Exploitation

Resistance to *A. flavus* in groundnut may operate at three sites in the plant—the pod, the seed coat, and the cotyledons. Zambettakis (1975) observed that the varieties Shulamit and Darou IV had lower levels of pod infection by *A. flavus* than other varieties tested in Senegal and attributed this to differences in pod-shell structure. Other workers have attributed resistance to the action of antagonistic microflora in the shell (Kushalappa et al. 1976), or to presence of thick-walled parenchyma cells (Pettit et al. 1977). After initial interest in the early 1970s only limited research on pod resistance has been reported.

Mixon and Rogers (1973) identified seed-coat resistance to *A. flavus* in the germplasm lines PI 337409 and PI 337394F by screening sound mature seeds of groundnut by artificial inoculation with *A. flavus* conidia in an environment favorable to *A. flavus* development. Subsequent reports confirmed seed-coat resistance in these lines and added several new germplasm lines and commercial varieties to the list of resistant materials. Among the reported resistant lines, the resistance in J 11, UF 71513, PI 337394F, PI 337409, Ah 7223, Faizpur 1-5, and Var.27 has been confirmed by testing over locations and years, but the stability of resistance in other lines has not been confirmed by multilocal testing. The lines with confirmed resistance have been used as gene donors for this characteristic. It has also been shown that resistance in J 11, PI 337394F, and PI 337409 can be transferred to other genetic backgrounds (Mixon 1986).

Genotypic differences are also reported for the ability of groundnut seeds to support the production of aflatoxins. Certain claims by earlier workers that US 26 (= PI 246388 = Koboka) and 'Asiriya Mwitunde' were resistant to aflatoxin production were not substantiated. However, Mehan et al. (1986) after screening over 500 genotypes, reported the low aflatoxin-producing ability in U 4-7-5 and VRR 245. No efforts are reported in the literature of genetically improving low aflatoxin-producing genotypes or transferring this trait to other lines. However, the two

germplasm lines reported to be low aflatoxin producers, provide us an opportunity of improving upon this trait and combining it with other *A. flavus* resistance traits.

Genetic variability has also been reported for resistance in groundnut which prevents the penetration and colonization by *A. flavus* in the field. The genotypes J 11, Ah 7223, UF 71513, and U 4-47-7 have been reported to be resistant to preharvest seed infection in India, while 55-437, PI 337409, 73-30, and 73-33 were reported to be resistant to seed infection by *A. flavus* in Senegal (Zambettakis et al. 1981). There has been only limited breeding effort to use preharvest resistance to seed infection as a selection trait. However, some of the sources of resistance to seed infection that also have seed-coat resistance, have been used in breeding programs to incorporate seed-coat resistance into high yielding cultivars.

It is interesting that although several factors such as; low testa permeability, increased surface wax accumulation, uniform wax coating, thin testa with compact and tight cell structure, compact palisade-like layer, small hilum, presence of tannins and inhibitory compounds, and differences in amino acid composition have been reported to contribute towards *A. flavus* resistance, no efforts have been made to breed for these traits. This may be because information on the contribution of these mechanisms to resistance traits is not fully available and/or they are highly influenced by environmental variations. There are no standard screening techniques for these mechanisms. More work will be required before the resistance mechanisms are understood.

Breeding for *A. flavus* Resistance at ICRISAT Center

Research is in progress at ICRISAT Center to incorporate seed-coat resistance into high yielding and adapted groundnut cultivars, and to study the genetics of seed-coat resistance. We are also exploring the possibilities of combining seed-coat resistance with low aflatoxin-producing ability, and hope to study the inheritance of low aflatoxin production.

Sources of Resistance and Crossing Plan

Genotypes used as parents in this breeding project have been selected based on the strength of their seed-coat resistance, and the stability of their resistance (Table 1). These genotypes have been used extensively as gene donors for seed-coat resistance. In addition, we have recently received genotypes AR 1, AR 3, and GFA 2 to be used as new sources of resistance; these have been multiplied and initial observations made to confirm their resistance. We have made crosses between resistant and adapted lines from important groundnut-growing countries where *A. flavus* infection and aflatoxin contamination are serious problems. We have also made crosses among source lines to bring together genes to strengthen resistance, assuming that different source lines possess non-allelic resistant genes.

Selection for Yield and Seed-coat Resistance

At ICRISAT Center, we follow a mass pedigree scheme to select for pod yield. In the F_2 generation, selection is based on the numbers of mature pods per plant. Progenies are advanced as bulks, and in each generation, selection is made for yield and other agronomic traits. In the F_6 - F_8 generations, bulks are separated based on the apparent uniformity for their plant and pod

Table 1. *Aspergillus flavus* seed colonization (%) and pod yield (kg ha⁻¹) of selected groundnut breeding lines in multilocal testing in India (1983–1986).

Identity	Pedigree	Postrainy season 1983/84		Rainy season			
				1984 ¹		1986 ²	
		SC (%)	Pod yield	SC (%)	Pod yield	SC (%)	Pod yield
ICGV 86168	(J 11 × PI 337394F)	15.24	5870	12.3	2420	9.17	1833
ICGV 86169	(PI 337409 × UF 71513)	11.62	4951	10.6	2294	10.31	1735
ICGV 86170	(Ah 32 × PI 337409)	14.36	5062	14.8	2336	16.87	1571
ICGV 86171	(J 11 × PI 337394F)	6.47	5796	9.6	1999	9.36	1617
ICGV 86173	(Faizpur 1-5 × PI 337409)	13.43	5407	12.4	2181	23.87	1586
ICGV 86174	(UF 71513 × PI 337394F)	11.71	5139	12.4	2262	10.21	1587
ICGV 87937	(NC 17 × PI 337394F)	16.40	4824	15.3	2225	NT	NT
ICGV 86177	(MH 2 × PI 337394F)	12.38	5302	14.9	2108	18.13	1740
Controls							
J 11 ³		12.88	5580	11.3	2077	9.69	1552
UF 71513 ³		11.58	5250	9.5	2151	9.71	1424
JL 24 ⁴		22.55	5262	39.2	2004	41.61	2001
Kadiri 3 ⁴		33.38	5000	31.1	2080	47.44	1570
Mean		16.33	5250	20.12– 24.70	707– 3269	15.0– 31.0	326– 2732
SE		±1.61	±270.1	±4.25– 6.01	±133.5– 446.6	±2.5– 6.2	±53.6– 464.4
CV (%)		17.1	8.9	33.8– 47.2	14.1– 20.6	27.7– 42.0	11.7– 31.2

1. Data mean from four locations; ICRISAT Center (High Input), ICRISAT Center (Low Input), Bhavanisagar, and Hisar.

2. Data mean from seven locations; ICRISAT Center (High Input Alfisol), ICRISAT Center (Low Input, Alfisol), ICRISAT Center (Low Input, Vertisol), Hisar, Dharwad, Bhavanisagar, and Anantapur.

3. *A. flavus* resistant varieties.

4. *A. flavus* susceptible varieties.

characters. These bulks are checked in the following generation for true-breeding character and uniformity. They are then entered into replicated yield trials. Harvested samples from these trials are sent to the laboratory where their seed-coat resistance is determined using the procedure described by Mehan et al. (1981), a modification of the procedure first described by Mixon and Rogers (1973).

If sufficient seed is available, breeding lines are tested in multilocal trials to evaluate the stability of their resistance. We emphasize the identification of stable resistance because past findings have indicated that environmental factors can influence seed-coat resistance (Diener et al. 1982, Sanders 1983, Davidson et al. 1983, Mehan et al. 1983).

In 1984, we modified the mass pedigree breeding system to include a stage of progeny-row testing in the F₃ generation, based on plant-to-row progenies obtained from selected F₂ plants. F₃ single-plant progenies are handled as progeny bulks from F₄ onwards and mass selection is made within each bulk. We plan to use a similar scheme to combine low aflatoxin production with seed-coat resistance. Because natural seed infection could be a better indicator of *A. flavus* resistance in the field, we are now planning to test breeding progenies in the field for preharvest seed infection by *A. flavus*.

Progress in Breeding

We have tested several hundred breeding lines for yield and seed-coat resistance. Generally, very few lines with *A. flavus* resistance and high yield have been recovered; this may be because of the low heritability of seed-coat resistance.

We now have eight breeding lines (Table 1) with seed-coat resistance levels equal to those of the resistant source lines J 11 and UF 71513. The yield levels in the selected lines, though fluctuating over the years, have been better than those of the resistant source lines. In a few years and locations, the resistant breeding lines have outyielded such susceptible commercial control varieties as JL 24 and Kadiri 3. Five lines, ICGV 86168, ICGV 86169, ICGV 86171, ICGV 86174, and ICGV 86177 are being evaluated in larger plots for seed infection and aflatoxin contamination.

We also have 32 breeding lines that have been tested once for seed-coat resistance. Some of these have high yield potential in addition to seed-coat resistance.

The importance of the stability of seed-coat resistance has been stressed by many previous workers. We have studied the stability of the resistant breeding lines, that were tested in the rainy seasons at four locations in 1984, and seven locations in 1986 (Tables 2 and 3), using the regression approach of Eberhart and Russell (1966). The regression coefficient (B_i) for resistance plotted against the mean percentage seed colonization (Fig 1, a and b) indicated that in both years, the selected resistant breeding lines were as stable as the resistant source lines and had similar levels of seed colonization to the resistance source lines. The regression coefficient (B_i) for yield plotted against the mean yield over locations indicated that some of the resistant breeding lines were also responsive to the environment.

Table 2. Stability parameters of eight breeding lines obtained from four Indian locations, rainy season, 1984.

Identity	Pod yield (kg ha ⁻¹)			Seed colonization (%)		
	Mean	B_i	S^2d_i	Mean	B_i	S^2d_i
ICGV 86168	2 420	1.088	376608	12.30	0.495	-17.8
ICGV 86169	2 294	1.060	-50440	10.65	0.288	-21.5
ICGV 86170	2 336	1.101	-22702	14.82	0.336	-20.2
GV 86171	1 999	0.991	62199	9.65	0.168	-11.2
GV 86173	2 181	1.138	46684	12.47	1.038	-5.4
ICGV 86174	2 262	0.896	54896	12.40	0.266	-19.9
ICGV 87937	2 225	0.910	54465	15.37	1.794	-22.6
ICGV 86177	2 108	1.008	-18571	14.93	1.007	-17.8
Controls						
J 11 ¹	2077	0.868	-36506	11.37	0.868	-26.1
UF 71513 ¹	2151	0.862	14654	9.50	0.606	-13.7
JL 24 ²	2004	1.040	-27055	39.20	2.565	20.0
Kadiri 3 ²	2080	0.833	113586	31.13	0.900	48.9

¹ Resistant control varieties.

² Susceptible control varieties.

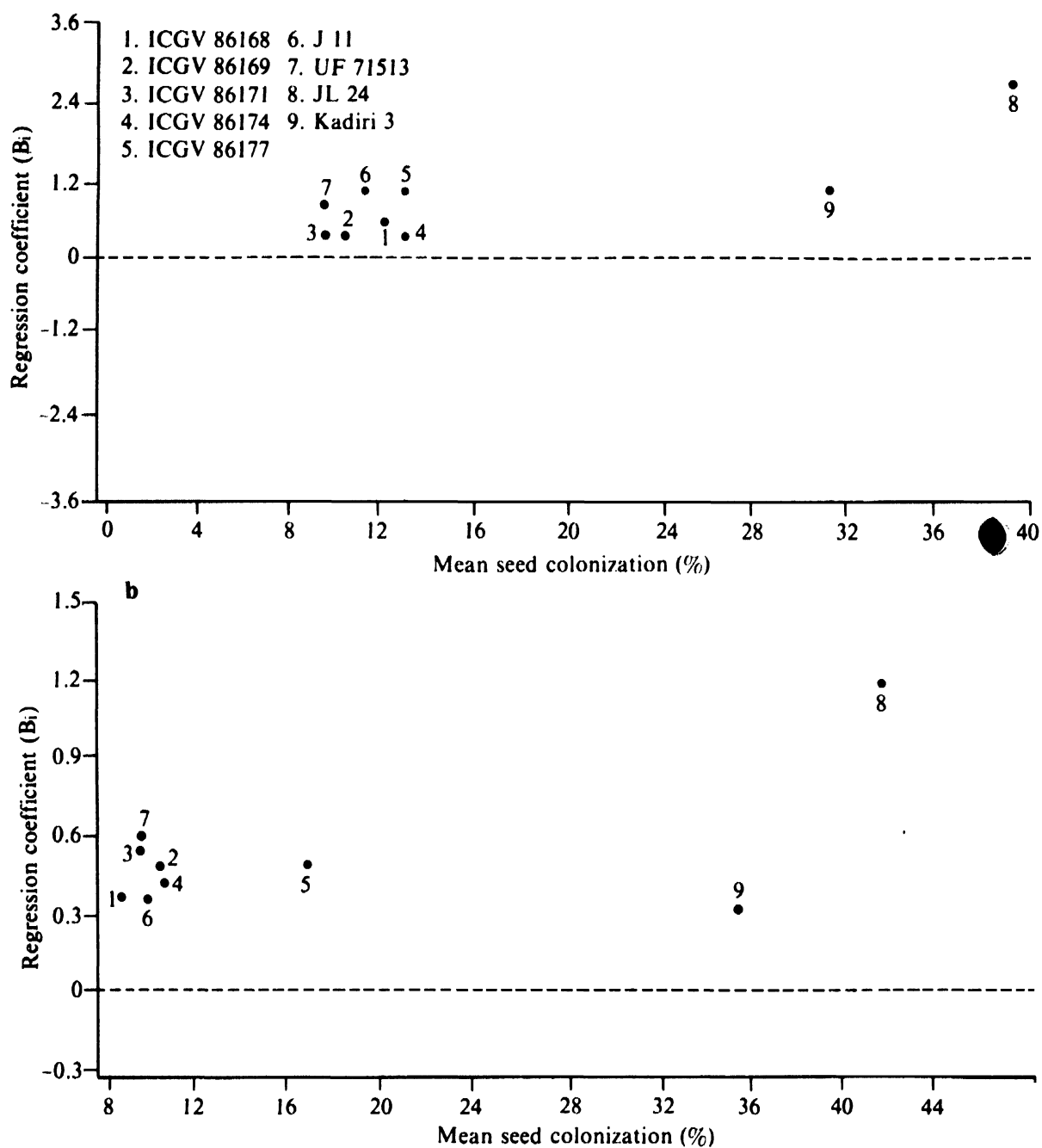


Figure 1. Stability of groundnut seedcoat resistance, ICRISAT Center. a. rainy season 1984, b. rainy season 1986.

Table 3. Stability parameters of six groundnut breeding lines obtained from seven Indian locations, rainy season 1986.

Identity	Pod yield (kg ha ⁻¹)			Seed colonization (%)		
	Mean	B _i	S ² d _i	Mean	B _i	S ² d _i
ICGV 86168	1833	1.173	76534	9.17	0.37	-1.74
ICGV 86169	1735	1.084	-3258	10.31	0.46	-3.65
ICGV 86170	1571	0.844	37960	16.87	0.50	38.26
ICGV 86171	1617	0.974	25471	9.36	0.56	-4.65
ICGV 86174	1587	0.783	187221	10.21	0.44	-2.73
ICGV 86177	1740	1.143	59424	18.13	0.83	11.93
Controls						
J 11 ¹	1552	0.765	56973	9.69	0.36	-2.87
UF 71513 ¹	1424	0.834	67347	9.71	0.59	-0.15
JL 24 ²	2001	1.229	-12013	41.61	1.21	23.01
Kadiri 3 ²	1570	0.810	115669	34.94	0.29	4.99

1. Resistant control varieties.

2. Susceptible control varieties.

Breeding for *A. flavus* Resistance at Other Locations

The group at Tifton, Georgia, USA lead by A.C. Mixon who first identified resistance in PI 337394F and PI 337409, have successfully transferred this resistance to other genetic backgrounds (Mixon 1983 a, 1983 b), producing the lines AR 1 to 4 which retain their resistance and yield more than their resistant parents in the USA. Breeding activities are in progress in Thailand (TCGIP 1985), and Senegal (Waliyar, Personal communication). At all the centers PI 337394F, PI 337409, UF 71513, and J 11 are common resistant parents in use as gene donors. In addition, scientists in Thailand have used AR 1 to 4 as new sources of resistance, and the variety 55-437 has been used in Senegal. A dry seed inoculation laboratory technique was used to screen selected lines in Thailand (Waranyuwat and Bhumibhaman 1985).

Genetics of Seed-coat Resistance

Of the different resistance traits, the genetics has been studied only for seed-coat resistance, and only one report (Mixon 1979) is available. This study, which evaluated the frequency distribution for percentage seed colonization from F₁ and F₂ plants of reciprocal crosses between PI 337409 (resistant) and PI 331326 (susceptible), indicated a broad sense heritability value of 78.5%. Subsequently, Mixon reported some more segregating population-evaluations to understand the genetics, but the conclusions were incomplete.

Investigations at ICRISAT Center have concentrated on understanding the combining abilities of the resistant parents. The main problem in the studies on inheritance of seed-coat resistance is the improper matching of the filial generations among testa, cotyledon, and embryo in the seed. In the seed of any of the filial generations, the testa belongs to the maternal generation while the cotyledons and embryo belong to the next generation. Thus, it has to be assumed that seed-coat resistance located in the testa is not influenced by the hybridity or

Table 4. General combining ability (GCA) effects for seven parental lines for seed-coat resistance in a F_0 line \times tester study, ICRISAT Center.

Parent	GCA effect
Testers	
Kadiri 3	+ 0.97
ICGS(AF)78	+ 1.97
U 4-7-5	- 2.95
Lines	
UF 71513	- 10.57
Ah 7223	- 20.62
J 11	- 1.82
Var. 27	+ 33.02
SE (Line)	\pm 1.49
SE (Tester)	\pm 1.72

Table 5. General combining ability (GCA) effects for four ground-nut lines for seed-coat resistance in a 4-parent F_0 and F_1 diallel, ICRISAT Center.

Parent	F_0 seed	F_1 seed
FESR-12- P_6 - B_1 - B_1	+27.04**	+26.85**
PI 337409	- 16.19**	- 8.84**
PI 337394F	- 6.88**	- 8.51**
UF 71513	- 2.61*	- 7.02**
Av. SE	\pm 1.03	\pm 1.13

Table 6. Reciprocal effects for six crosses for seed-coat resistance in a 4-parent F_0 and F_1 diallel, ICRISAT Center.

Cross ¹	F_0 seed	F_1 seed
$P_1 \times P_2$	- ²	+12.37**
$P_1 \times P_3$	+ 0.41	+ 5.08*
$P_1 \times P_4$	+34.92**	- ¹
$P_2 \times P_3$	-0.45	-1.67
$P_2 \times P_4$	+32.86**	+18.68**
$P_3 \times P_4$	+ 5.08	-0.41
Av. SE	\pm 2.14	\pm 2.34

1. P_1 = FESR-12- P_6 - B_1 - B_1 , P_2 = PI 337409, P_3 = PI 337394F, and P_4 = UF 71513.

2. Reciprocal cross missing.

heterosis exhibited by the cotyledon/embryo. Preliminary studies on combining ability using line \times tester analysis on F_0 seed indicated that UF 71513 and Ah 7223 had significant negative GCA effects and therefore were good combiners for seed-coat resistance (Table 4). Variety J 11 registered a nonsignificant GCA effect. Var. 27 turned out to be a poor combiner. The F_0 and F_1 diallel study (Table 5) also indicated PI 337409, PI 337394F, and UF 71513 to be good combiners for seed-coat resistance. Significant reciprocal effects were noticed in some crosses both in F_0 and F_1 for seed-coat resistance (Table 6), perhaps because of the significant maternal influence on testa structure.

Problems in Breeding for *A. flavus* Resistance and Future Priorities

The Resistance Trait

At least three possibly interdependent resistance traits are known to operate but exact information on their relationships, interactions, and their possible contributions to reducing aflatoxin contamination of groundnut are not clearly established. Their relationships with preharvest natural seed infection and infections during postharvest handling and storage are not fully understood. Research is required to understand these traits and their interdependence, so that breeding activities can be properly focused.

Environmental Influences on Resistance Traits

Two questions arise concerning environmental influences on resistance traits; on the usefulness of breeding for these traits, and on the problem of the extensive sampling required to confirm the stability of resistance. Efforts are required to strengthen the sources of resistance by crossing lines with different resistance traits and bringing together the different resistance genes (assuming that the resistance genes are non-allelic).

Screening Techniques

Currently available screening techniques for low aflatoxin production are expensive. Cheaper and more reliable techniques are needed. Techniques to screen single plants for all the resistance traits should be developed.

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Discussion

B. Singh. How long does it take to identify a resistant line?

M.J. Vasudeva Rao. It may take a long time to identify a genotype with seed resistance to infection by *A. flavus* as this depends on the heritability of the trait, environmental influence, screening facilities available, etc. We do, however, know from work over the past 15-20 years that resistance does exist in some genotypes. To breed a cultivar with acceptable agronomic traits could take several years if there were significant deficiencies in the quality and yielding ability of the resistant source line.