

Aflatoxin production in seeds of wild *Arachis* species(1)

V.K. MEHAN⁽²⁾, D. McDONALD⁽²⁾, A.K. SINGH⁽³⁾, J.P. MOSS⁽²⁾

Summary — Twenty-two accessions of wild *Arachis* species and five of groundnut (*Arachis hypogaea* L.) were tested for aflatoxin production. All *Arachis* accessions and groundnut cultivars supported production of aflatoxin B₁. *A. duranensis* and *A. cardenasii*, earlier reported to possess near absolute resistance to aflatoxin production, supported substantial amounts of aflatoxin B₁ but significant differences in levels of aflatoxin production were found between the accessions of the species tested. The groundnut genotype OG 35 1 previously reported to support low level of aflatoxin production was found to support substantial level of aflatoxin production.

Key words — *Arachis* species, *Arachis hypogaea*, aflatoxin B₁, *Aspergillus flavus*

INTRODUCTION

Researchers previously reported resistance of groundnut cultivars to aflatoxin production when seeds were colonized by aflatoxin-producing strains of *Aspergillus flavus* Link ex Fries and *A. parasiticus* Speare (Rao and Tulpule, 1967, Kulkarni *et al.*, 1967). Although reports of absolute inhibition of aflatoxin production in the cultivated groundnut (*Arachis hypogaea* L.) have not been confirmed, significant quantitative genotypic differences in aflatoxin production were found (Doupnik, 1969, Mehan *et al.*, 1986, Kalia *et al.*, 1988). Recently, two wild *Arachis* species, *Arachis cardenasii* and *A. duranensis*, have been reported to support only trace levels of aflatoxins (Ghewande *et al.*, 1989). This paper reports *in vitro* production of aflatoxin in seeds of various wild *Arachis* species.

MATERIALS AND METHODS

Wild *Arachis* species/groundnut genotypes. 22 accessions of *Arachis* species and five groundnut genotypes were tested. Seeds of these accessions/genotypes were obtained from the ICRISAT Genetic Resources Unit.

Aflatoxin production tests: aflatoxin production tests were carried out on seeds of *Arachis* species/groundnut genotype by the method described by Mehan and McDonald (1980). Undamaged, mature seeds of each accession/cultivar were surface-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 seed lot to increase the seed moisture content to 20%. The seeds were then placed in Petri plates, and their testae scarified with sterile needles, and 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 *A. flavus*. Three replicates were used for each species/genotype. Plates were incubated at 25°C for 10 days. Plates were arranged in a completely randomized design in an incubator. After incubation, aflatoxins were determined by the method of Pons *et al.* (1966).

The mean aflatoxin production levels were estimated with their standard error using analysis of variance.

RESULTS

All *Arachis* accessions and cultivars supported aflatoxin B₁ production, but there were significant ($P=0.01$) genotypic differences in aflatoxin production (Table 1). The commonly cultivated Indian Cultivar TMV₂ supported the highest level of aflatoxin B₁ production (196 µg g⁻¹ seed). The other *A. hypogaea* cultivars and a few accessions of *A. batizocoi*, *A. duranensis* and *A. paraguariensis* supported high levels of aflatoxin (93.3 - 110.0 µg g⁻¹ seed). Most accessions of *Arachis* species supported production of between 50 and 80 µg of aflatoxin B₁ g⁻¹ of seed. There was considerable variation among accessions of the same *Arachis* species for support to aflatoxin production by the aflatoxigenic fungus. No aflatoxins were found in non-inoculated seeds of any *Arachis* accession/cultivar.

DISCUSSION

We were unable to confirm the previous report that indicated near absolute inhibition of aflatoxin production in seeds of *A. duranensis* and *A. cardenasii* (Ghewande *et al.*, 1989). Nevertheless, the levels of aflatoxin production were comparatively lower in seeds of most wild *Arachis* species than the cultivars of *A. hypogaea* tested. However, the present studies indicate the presence of variability between the accessions of an *Arachis* species for supporting production of aflatoxin. This suggests that further exploration in such potential species may result in identification of accessions that support only very low levels of aflatoxin. This

(1) Submitted as Journal Article N° 1171 by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)

(2) Legumes Program, ICRISAT, Patancheru P.O., Andhra Pradesh 502 324, India

(3) Genetic Resources Unit, ICRISAT, Patancheru P.O., Andhra Pradesh 502 324, India

TABLE I. — Aflatoxin B₁ production in seed of *Arachis* species and *Arachis hypogaea* genotypes.

ICG N°	<i>Arachis</i> species	Collection N°	Section	Series	Country of origin	Aflatoxin B ₁ production (µg g ⁻¹ seed)
8124	<i>A. butisocoi</i>	9484	Arachis	Annuae	Bolivia	103.3
8958	<i>A. butisocoi</i>	30080	Arachis	Annuae	Bolivia	65.0
8123	<i>A. duranensis</i>	7988	Arachis	Annuae	Argentina	110.0
11555	<i>A. duranensis</i>	36005	Arachis	Annuae	Argentina	21.6
8956	<i>A. duranensis</i>	30065	Arachis	Annuae	Argentina	35.0
8202	<i>A. duranensis</i>	30070	Arachis	Annuae	Bolivia	35.0
8139	<i>A. spegazzinii</i>	10038LL	Arachis	Annuae	Argentina	60.0
8138	<i>A. spegazzinii</i>	10038SL	Arachis	Annuae	Argentina	66.6
8960	<i>A. sp</i>	30092	Arachis	Annuae	Bolivia	38.0
11564	<i>A. cardenusii</i>	36034	Arachis	Perennes	Bolivia	68.7
8216	<i>A. cardenusii</i>	10017	Arachis	Perennes	Bolivia	34.1
4983	<i>A. chucoense</i>	10602	Arachis	Perennes	Paraguay	55.0
8955	<i>A. helodes</i>	30036	Arachis	Perennes	Brazil	60.0
11549	<i>A. monticola</i>	30063	Arachis	Amphiploides	Argentina	80.0
8970	<i>A. paraguariensis</i>	30124	Erectoides	Tetrafoliatae	Paraguay	50.0
8973	<i>A. paraguariensis</i>	30134	Erectoides	Tetrafoliatae	Brazil	93.3
8945	<i>A. apressipila</i>	30003	Erectoides	Procumbensae	Brazil	85.0
8946	<i>A. apressipila</i>	30009	Erectoides	Procumbensae	Brazil	52.8
8923	<i>A. glabrata</i>	489	Rhizomatosaes	Eurhizomatosaes	Brazil	55.0
8933	<i>Arachis sp.</i>	9797	Rhizomatosaes	Eurhizomatosaes	Brazil	80.0
8142	<i>A. villosulicarpa</i>	-	Extranervosaes	-	Brazil	61.6
8131	<i>A. triseminalis</i> ⁽¹⁾	12922	Triseminalae	-	Brazil	65.0
221	<i>A. hypogaea</i>	TMV2 (Spanish)	Arachis	Amphiploides	India	196.6
2738	<i>A. hypogaea</i>	Gangapuri (Valencia)	Arachis	Amphiploides	India	146.6
799	<i>A. hypogaea</i>	Robut 33-1 (Virginia bunch)	Arachis	Amphiploides	India	106.6
156	<i>A. hypogaea</i>	M13 (Virginia bunch)	Arachis	Amphiploides	India	106.6
7829	<i>A. hypogaea</i>	OG 35-1 (Spanish)	Arachis	Amphiploides	India	80.8
SE						± 9.43

(1) Previously cited as *A. pusilla*.

also emphasizes that identity of the accessions tested should be given for validation of results and their utilization in a breeding program.

A. hypogaea, OG 35-1 was previously reported to support low levels of aflatoxin production (Kalia *et al.*, 1988). However, in the present study OG 35-1 supported substantial levels of

aflatoxin production. The present study substantiates the genetic variability of aflatoxin production in the genus *Arachis*. Wild *Arachis* species may be useful in developing genotypes that inhibit aflatoxin production. We plan to test all the available *Arachis* accessions at ICRISAT.

REFERENCES

- [1] DOUPNIK B., Jr. (1969). — Aflatoxins produced on peanut varieties previously reported to inhibit production. *Phytopath.*, 59, 1554.
- [2] GHEWANDE M.P., NAGARAJ G. and REDDY P.S. (1989). — Aflatoxin research at the Indian National Research Center for groundnut. In: Aflatoxin contamination of groundnut: proceedings of the International Workshop, 6-9 oct. 1987, ICRISAT Center, India, Patancheru, Andhra Pradesh 502 324, India: ICRISAT, p. 237-243.
- [3] KALIA K., DESAI H.M. and CHAKRABORTY M.K. (1988). — Resistance of groundnut (*Arachis hypogaea*) to aflatoxin. *Indian J. Agric. Sci.*, 58, 121-123.
- [4] KULKARNI L.G., SHARIEF Y. and SARMA V.S. (1967). — "Asrya Mwitunde" groundnut gives results in Hyderabad. *Indian Farming*, 17, 11-12.
- [5] MEHAN V.K. and McDONALD D. (1980). — Screening for resistance to *Aspergillus flavus* invasion and aflatoxin production in groundnuts. ICRISAT Groundnut Improvement Program Occasional paper n°2. Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics. 15 p. (Limited distribution).
- [6] MEHAN V.K., McDONALD D. and RAMAKRISHNA N. (1986). — Varietal resistance in peanut to aflatoxin production. *Peanut Sci.*, 13, 7-10.
- [7] PONS W.A., CUCULLU A.F., LEE L.S., FRANZA A.O. and GOLDBLATT L.A. (1966). — Determination of aflatoxins in agricultural products: use of aqueous acetone for extraction. *J. Assoc. Off. Anal. Chem.*, 49, 554-562.
- [8] RAO K.S. and TULPULE P.G. (1967). — Varietal differences of groundnut in the production of aflatoxin. *Nature*, 214, 738-739.

RESUME

Production d'aflatoxines dans les graines d'espèces spontanées d'*Arachis*

V.K. MEHAN, D. McDONALD, A.K. SINGH, J.P. MOSS, *Oléagineux*, 1992, 47, N° 2, p. 87-89.

La production d'aflatoxines a été étudiée chez vingt-deux introductions d'espèces spontanées d'*Arachis* et chez cinq cultivars d'arachide (*Arachis hypogaea* L.). Toutes les accessions d'*Arachis* et tous les cultivars d'arachide présentaient une production d'aflatoxine B₁. Les espèces *A. duranensis* et *A. cardenasii*, dont la résistance quasi absolue à la production d'aflatoxines avait été signalée auparavant, présentaient des teneurs importantes en aflatoxine B₁, mais des différences significatives ont été observées entre les accessions des espèces testées en ce qui concerne les taux de production d'aflatoxines. Dans le cas du génotype d'arachide OG 35-1, dont on avait signalé auparavant le faible taux de production d'aflatoxines, ce taux s'est avéré important.

Mots clés. — Espèces d'*Arachis*, *Arachis hypogaea*, aflatoxine B₁, *Aspergillus flavus*

RESUMEN

Producción de aflatoxinas en las semillas de especies espontaneas de *Arachis*

V.K. MEHAN, D. McDONALD, A.K. SINGH, J.P. MOSS, *Oléagineux*, 1992, 47, N° 2, p. 87-89.

Se probaron veintidos introducciones especies espontaneas de *Arachis* y cinco cultivares de maní (*Arachis hypogaea* L.) para estudiar la producción de aflatoxinas. Todas las incorporaciones de *Arachis* y todas las variedades del maní presentaban una producción de aflatoxina B₁. Las especies *A. duranensis* y *A. cardenasii*, cuya resistencia casi absoluta a la producción de aflatoxina fue señalada anteriormente, presentaban contenidos importantes de aflatoxina B₁, pero se observaron diferencias significativas entre incorporaciones de especies probadas referente a las tasas de producción de aflatoxina.

En el caso del genotipo del maní OG 35-1, cuya baja tasa de producción de aflatoxina fue señalada con anterioridad, esta tasa reveló ser importante.

Palabras-claves. — Especies de *Arachis*, *Arachis hypogaea*, aflatoxina B₁, *Aspergillus flavus*