A Note on Use of Seed Protein Markers for Identification of Aflatoxin Resistance in Peanut[1]

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ABSTRACT

Fungi in the genus *Aspergillus* produce aflatoxins which are a group of toxic secondary metabolites. Fungal invasion of peanut seed and subsequent aflatoxin production can occur before or during harvest as well as during storage. Because storage proteins comprise a large percentage of the peanut seed, this study attempted to associate protein markers with previously reported aflatoxin-resistant genotypes. Variation was observed among 24 genotypes for electrophoretic banding patterns, but it was not possible to correlate the presence or absence of specific bands with aflatoxin resistance.

Key Words: *Arachis hypogaea*, groundnut, *Aspergillus* spp., aflatoxins, seed storage proteins.

Fungi in the genus *Aspergillus* produce aflatoxins as secondary metabolites which are highly poisonous, teratogenic, and carcinogenic (Wogan and Pong, 1970; Heathcote and Hibbert, 1978). Among the most widely distributed *Aspergillus* species that infect oil seed and cereal crops are *A. flavus* Link ex Fries and *A. parasiticus* Speare. Different strains of these species produce varying types and levels of aflatoxins (Diener *et al.*, 1982).

The invasion of peanut by *Aspergillus* occurs any time during seed development, harvest, and storage. Suppression of toxin accumulation can be accomplished if plants have preharvest resistance to infection, dry seed resistance to pathogen invasion during storage, or if seeds inhibit toxin production when the pathogen is present. Variability among peanut cultivars for resistance to *Aspergillus* spp. has been reported by Mixon and Rogers (1973), Davidson *et al.* (1983), Mixon (1983a,b), Blankenship *et al.* (1985), Mehan *et al.* (1988), Azaizeh *et al.* (1989), Pettit *et al.* (1989), Vasudeva Rao *et al.* (1989), Szerszen and Pettit (1990), Utomo (1990), Holbrook *et al.* (1992), and Waliyar *et al.* (1994) (Table 1).

Infection by *Aspergillus* fungi and toxin production are processes that occur in the peanut seed. Screening genotypes for aflatoxin resistance in the field or greenhouse is time-consuming and expensive, and alternative approaches to identify resistant genotypes are needed. Because storage proteins comprise approximately 70% of the total nitrogen in peanut seeds, and many differences in electrophoretic profiles have been observed among *Arachis* species (Bianchi-Hall *et al.*, 1992), it may be possible to identify protein markers in resistant peanut genotypes. The objective of this study was to associate seed storage protein markers in peanut with aflatoxin resistance.

Table 1. Aflatoxin reaction reported in the literature for genotypes studied and 30-kD band observed.

Genotype	Seed source	*Asp.* spp.[a] reaction	Citation	30 kD band[b]
Ah 7223	NCSU	R	Mehan *et al.*, 1988	-
AR-1	USDA	R	Mixon, 1983b	-
AR-2	USDA	R	Mixon, 1983b	-
AR-3	USDA	R	Mixon, 1983b	-
AR-4	USDA	R	Mixon, 1983b	+
C55-437	NCSU	R	Mehan *et al.*, 1988	-
Faizpur	NCSU	R	Mehan *et al.*, 1988	-
GFA-1	NCSU	R	Mixon, 1983a	+
GFA-2	NCSU	R	Mixon, 1983a	+
J-11	NCSU	R	Mehan *et al.*, 1988; Szerszen & Pettit, 1990	-
Monir 240-30	NCSU	R	Mehan, 1989; Utomo, 1990	+
PI 337409	USDA	R	Szerszen & Pettit, 1990; Mixon & Rogers, 1973	-
PI 337394(F)	NCSU	R	Mehan *et al.*, 1988; Mixon & Rogers, 1973	-
Sunbelt Runner	NCSU	R	Davidson *et al.*, 1983	+
U4-47-7	NCSU	R	Mehan *et al.*, 1988	-
UF 71-513	NCSU	R	Mehan *et al.*, 1988	-
Var. 27	NCSU	R	Mehan *et al.*, 1988	-
Florunner	NCSU	S	Szerszen & Pettit, 1990; Mixon & Rogers, 1973; Mixon, 1983b	+
NC 7	NCSU	S		+
(NC 7 x AR-4) 89-03	NCSU	R	Utomo, 1990	+
(NC 7 x AR-4) 89-05	NCSU	R	Utomo, 1990	+
(NC 7 x AR-4) 89-08	NCSU	R	Utomo, 1990	+
(NC 7 x GFA-2) 89-59	NCSU	R	Utomo, 1990	-

[a] *Aspergillus* reaction rating: R = resistant, S = susceptible.

[b] + = present, - = absent.

[1]This research was partially supported by the North Carolina Agric. Res. Serv., Raleigh, NC 27695 and the Peanut CRSP, USAID grant number DAN-4048-G-SS-2065-00. Recommendations neither represent an official position nor policy of the NCARS or USAID.

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Materials and Methods

The seed storage protein profiles of 22 *A. hypogaea* L. genotypes, which have been reported as having aflatoxin resistance, plus cv. NC 7 were analyzed by SDS-PAGE (Table 1). Seeds of six genotypes—PI 337409, PI 363058, AR-1, AR-2, AR-3, and AR-4—were obtained from Dr. R. Pittman, USDA Peanut Curator, Griffin. GA. Seeds of three lines derived from the cross (NC 7 x AR-4) and from another line derived from the cross (NC 7 x GFA-2) were supplied by Dr. T. G. Isleib, NCSU, Raleigh, NC. All other genotypes were maintained in the peanut breeding program and harvested at the Peanut Belt Research Station, Lewiston, NC in 1991.

Protocols for extraction of seed storage proteins, preparation of electrophoresis samples, and other procedures used were described by Bianchi-Hall *et al.* (1992). Three g of seeds were used in each of two replications and two duplicate samples per genotype were run on gels. Molecular weight standard proteins in the range of 2 to 66 kD were used as molecular weight reference markers.

Results and Discussion

Variability was observed among the seed storage protein profiles of the 23 genotypes (Fig. 1). Many patterns were common to more than one genotype—*i.e.*, GFA-1, GFA-2, and cv. Sunbelt Runner. All the AR- genotypes had very similar protein profiles. AR-1 and AR-4 could be separated from the other two genotypes because of the presence of an acidic arachin protein band of approximately 44 kD, and AR-1 was missing an intermediate molecular weight protein at about 30 kD which is present in AR-4. PI 337409 was undifferentiated from AR-2. PI 337394, C 55-437, cv. Faizpur, cv. J-11, Var. 27 (not shown), and UF 71-513 had similar protein profiles (Fig. 1A). Monir 240-30 had a protein profile similar to these five genotypes, except it also had a 30-kD band. U4-47-71 had a protein profile different from other genotypes, with four arachin and several unique intermediate bands (Fig. 1A).

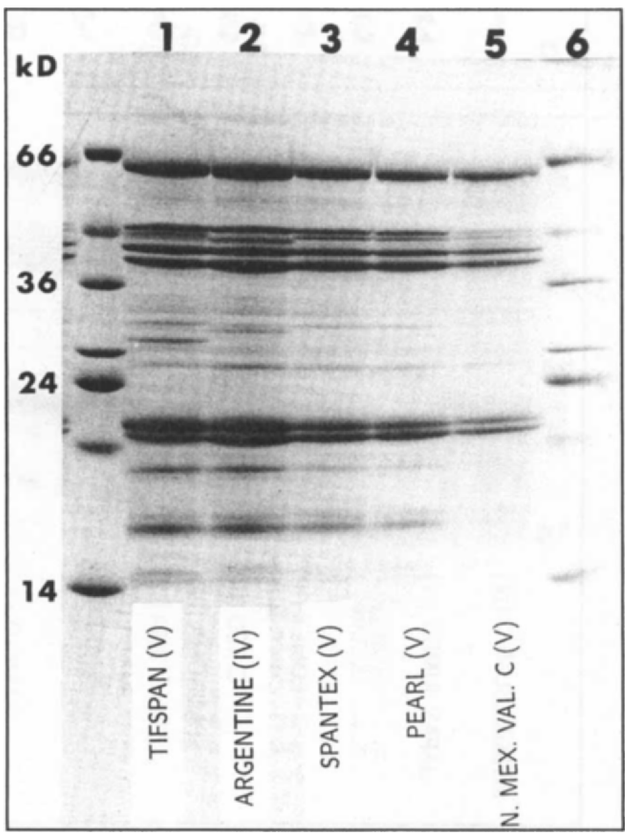

Fig. 1. SDS-PAGE seed storage protein profiles of genotypes as follows (arrow indicates position of 30-kD band): (A) 1, GFA-1; 2, GFA-2; 3, NC 7; 4, AR-4; 5, PI 337394; 6, C55-437; 7, J-11; 8, Faizpur; 9, Monir 240-30; 10, Sunbelt Runner; 11, U4-47-1; 12, UF 71-513; 13, molecular weight standards. (B) 1, Ah-7223; 2, AR-1; 3, AR-2; 4, AR-3; 5, AR-4; 6, PI 337394; 7, GFA-2; 8, (NC 7 x GFA-2)-89-59; 9, NC 7; 10, (NC 7 x AR-4)-89-05; 11, (NC 7 x AR-4)-89-03; 12, (NC 7 x AR-4)-89-08; 13, Florunner; 14, molecular weight standards.

NC 7 had a similar profile to AR-4 (Fig. 1B), thus it was not possible to trace the inheritance of unique bands in progeny of a cross between the genotypes. However, NC 7 and GFA-2 had several band differences; progeny of this cross had a protein profile different from either of its parents by not having an intermediate molecular weight protein band at about 30 kD. This is the same band which was absent in PI 337394, C55-437, Faizpur, J-11, UF 71-513, and AR-1.

Although a large amount of variability was observed among the protein profiles of the 23 peanut genotypes, it was not possible to correlate particular protein bands (or their absence) with aflatoxin resistance. Furthermore, when an attempt was made to study the protein profiles of several hybrids, no differences were observed, indicating that the susceptible NC 7 was not different from resistant genotypes or their progenies based upon SDS-PAGE. A single denominator of either presence or complete absence of a protein band could not be found in all SDS protein profiles of aflatoxin-resistant genotypes. However, the 30-kD band was absent in about 60% of the resistant genotypes (67% of genotypes not having NC 7 in the pedigree) analyzed and possibly may be associated with *Aspergillus* infection resistance or to toxin production.

Because a high correlation between SDS protein profiles and aflatoxin resistance was not found, it appears that one-way SDS electrophoresis is not a sufficiently powerful technique to be used to identify markers for aflatoxin resistance in peanut. Alternatives to this conclusion are that seed storage proteins do not have anything to do with resistance or that the genotypes reported in the literature are not truly resistant. Further, Waliyar *et al.* (1994) reported that at least some genotypes reported in this paper as resistant [e.g., C 55-437, J-11, and PI 337394(F)] to be significantly more resistant than other genotypes. When Szerszen and Pettit (1990) used SDS-PAGE two-dimensional electrophoresis in combination with silver staining, they identified several unique polypeptides in the second-dimension gel produced by the aflatoxin-resistant cultivar TX 798736. Unfortunately, the synthesis of polypeptides varied over time and in quantity among the 14 cultivars that they tested. Several mechanisms of resistance appear to be present in the germplasm collection of *A. hypogaea*, but identification of aflatoxin-resistant genotypes by using seed storage protein markers is not possible at this time.

Literature Cited

1. Azaizeh, H. A., R. E. Pettit, O. D. Smith, and R. A. Taber. 1989. Reaction of peanut genotypes under drought stress to *Aspergillus*

flavus and *A. parasiticus*. Peanut Sci. 16:109-113.
2. Bianchi-Hall, C. M. 1992. Seed storage proteins in peanuts associated with the characterization of species, cultivars and molecular markers. PhD Thesis, North Carolina State Univ., Raleigh, NC. 133 pp.
3. Bianchi-Hall, C. M., R. D. Keys, H. T. Stalker, and J. P. Murphy. 1993. Diversity of seed storage protein patterns in wild peanuts (*Arachis* species). Plant Evol. Syst. 186:1-15.
4. Blankenship, P. D., R. J. Cole, and T. H. Sanders. 1985. Comparative susceptibility of four experimental peanut lines and the cultivar Flo runner to preharvest aflatoxin contamination. Amer. Peanut Res. Educ. Soc. Proc. 17:72 (abstr.).
5. Davidson, J. I., Jr., R. A. Hill, R. J. Cole, A. C. Mixon, and R. J. Henning. 1983. Field performance of two peanut cultivars relative to aflatoxin contamination. Peanut Sci. 10:43-47.
6. Diener, U. L., R. E. Pettit, and R. J. Cole. 1982. Aflatoxins and other mycotoxins in peanuts, pp. 486-519. *In* H. E. Pattee and C. T. Young (eds.), Peanut Science and Technology. Amer. Peanut Res. Educ. Soc., Yoakum, TX.
7. Heathcote, J. G., and J. R. Hibbert. 1978. Pathological effects, pp. 83-111. *In* Developments in Food Science. I. Aflatoxins: Chemical and Biological Aspects. Elsevier Scien. Publ. Co., Amsterdam, The Netherlands.
8. Holbrook, C. C., D. M. Wilson, W. F. Anderson, M. E. Will, and M. E. Matheron. 1992. Improvements in screening techniques for resistance to preharvest aflatoxin contamination and some potential sources of resistance. Amer. Peanut Res. Educ. Soc. Proc. 24:23 (abstr.).
9. Mehan, V. K., R. C. Nageswara Rao, D. McDonald, and J. H. Williams. 1988. Management of drought stress to improve field screening of pea nuts for resistance to *Aspergillus flavus*. Phytopathology 78:659-663.
10. Mixon, A. C. 1983a. Peanut germplasm lines, AR-1, -2, -3, and -4. Crop Sci. 23:1021.
11. Mixon, A. C. 1983b. Peanut germplasm lines, GFA-1 and TFA-2. Crop Sci. 23:1020-1021.
12. Mixon, A. C., and K. M. Rogers. 1973. Peanut accessions resistant to seed infection by *Aspergillus flavus*. Agron. J. 65:560-562.
13. Pettit, R. E., H. A. Azaizeh, R. A. Taber, J. B. Szerszen, and O. D. Smith. 1989. Screening groundnut cultivars for resistance to *Aspergillus flavus, Aspergillus parasiticus* and aflatoxin contamination, pp. 291-315. *In* Aflatoxin Contamination of Groundnut, Proc. of the International Workshop, 6-9 Oct. 1987. International Crops Research Institute for the Semi-Arid Tropics, ICRISAT Center, Patancheru, India.
14. Szerszen, J. B., and R. E. Pettit. 1990. Detection and partial characterization of new polypeptides in peanut cotyledons associated with early stages of infection by *Aspergillus* spp. Phytopathology 80:1432-1438.
15. Utomo, S. D. 1990. Inheritance of resistance to *Aspergillus parasiticus* Speare in peanut (*Arachis hypogaea* L.). Unpub. M.S. Thesis, Dept. of Crop Science, North Carolina State Univ., Raleigh.
16. Vasudeva Rao, M. J., S. N. Nigam, V. K. Mehan, and D. McDonald. 1989. *Aspergillus flavus* resistance breeding in groundnut: Progress made at ICRISAT Center, pp. 345-355. *In* Aflatoxin Contamination of Groundnut, Proc. of the International Workshop, 6-9 Oct. 1987. International Crops Research Institute for the Semi-Arid Tropics, ICRISAT Center, Patancheru, India.
17. Waliyar, F., H. Hassan, and J. P. Bosc. 1994. Sources of resistance to *Aspergillus flavus* and aflatoxin contamination in groundnut genotypes in West Africa. Plant Dis. 78:704-708.
18. Wogan, G. N., and R. S. Pong. 1970. Aflatoxins. Ann. N.Y. Acad. Sci. 174:623-635.

Accepted November 14, 1994