Polyphenols in Groundnut Genotypes Resistant and Susceptible to Seed Colonization by *Aspergillus flavus*

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Abstract

Thirteen groundnut genotypes, eight resistant and five susceptible to in vitro seed colonization by Aspergillus flavus *were grown in replicated trials at three locations in Andhra Pradesh, India. Seed coats of these genotypes were analyzed for polyphenols using different methods. No significant correlation was observed between seed colonization and polyphenols content, which corroborates earlier observations on many genotypes using a single method for polyphenols estimation.*

Résumé

Les polyphénols dans les génotypes d'arachide résistants ou sensibles à la colonisation des graines par *Aspergillus flavus*: *Treize génotypes d'arachide, dont huit résistants et cinq sensibles à la colonisation in vitro des graines par* Aspergillus flavus *ont été étudiés dans le cadre d'essais répétés sur trois sites en Andhra Pradesh (Inde). Les téguments de ces génotypes ont été analysés par différentes méthodes pour la présence des polyphénols. On n'a observé aucune corrélation significative entre la colonisation et la teneur en polyphénols, ce qui corrobore une observation antérieure faite sur plusieurs génotypes en utilisant une seule méthode d'estimation des polyphénols.*

Resúmene

Los polifenoles presentes en los genotipos de cacahuate resistentes y susceptibles a la colonización de las semillas por *Aspergillus flavus*: *Trece genotipos de cacahuates, ocho resistentes y cinco susceptibles a la colonización de la semilla* in vitro *por* Aspergillus flavus, *se evaluaron en pruebas replicadas en tres localidades de Andhra Pradesh, India. Los tegumentos de las semillas de estos genotipos se analizaron para determinar su contenido de polifenoles, usando diferentes métodos. No se observó una correlación significativa entre la colonización de las semillas y su contenido de polifenoles, lo cual corrobora observaciones anteriores hechas en gran número de genotipos, usando un solo método de cuantificación de polifenoles.*

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Introduction

Tannins, which are polyphenols, may be defined as water-soluble phenolic metabolites of plants, with molecular weight of 500 or more, and with the ability to bind to and/or precipitate gelatin and/or other proteins from aqueous solutions (Butler In press). Tannins isolated from groundnut seed coat produce cyanidin during acid hydrolysis and therefore belong to the class of condensed procyanidins (Lansden 1982). Turner et al. (1975) isolated 5-7-dimethoxyisoflavone from groundnut and observed that it inhibited the growth of *Aspergillus flavus* Link ex Fries. Sanders and Mixen (1978) reported a significant and negative correlation between percentage seed colonization by *Aspergillus parasiticus* Speare and tannin content in groundnuts. Amaya et al. (1980) reported a possible relationship between resistance to *A. flavus* and total soluble amino compounds and arabinose content in groundnut cultivars. Lansden (1982) isolated three fractions of tannins from groundnut testae and observed that the fractions inhibited the growth of *A. parasiticus*. Karchesy and Hemingway (1986) have identified various compounds that are present in groundnut seed coats and determined the structure of some of the isolated polyphenolic compounds. We examined polyphenol content of seed coats in relation to seed colonization by *A. flavus* in several groundnut genotypes.

Materials and Methods

Fifty groundnut genotypes grown at ICRISAT Center in the 1982 rainy season, and 13 grown in replicated trials at three locations (ICRISAT Center, Tirupati, and Anantapur) in Andhra Pradesh State, India, in the 1985 rainy season were used in this study. The genotypes were tested for their reactions to in vitro seed colonization by *A. flavus* (Mehan and McDonald 1980).

Sample Extracts

Seed coats of groundnut genotypes were ground to a fine powder in a PICA® mill (Pitchford Industries, Pittsburg, USA), and defatted with n-hexane in a Soxhlet apparatus. The samples were air dried and then further dried at 37°C for 2 h. Ground seed coat (100 mg) was extracted with 5 mL methanol for 1 h in a test-tube mixer (Stuart Tube Rotator TR-2®, UK). After centrifuging, the supernatant was saved and the residue was reextracted with 5 mL methanol for 30 min and the methanol extracts were pooled together. The residue was extracted with 5 mL methanol containing 1% concentrated hydrochloric acid (vol/vol) and after centrifuging, this procedure was repeated and the extracts were pooled together. Both the methanol and acidified methanol extracts were used for the estimation of polyphenols. Analyses were carried out on individual replicate seed-coat samples from two locations as well as on pooled samples from all three locations. Results obtained on pooled samples are reported here as the agreement between the values obtained on individual replicate and pooled data was excellent.

Polyphenol Estimation

Total phenols were estimated using the modified Prussian blue method (Butler 1982). Total phenols were also extracted independently from each seed coat sample using acidified methanol extract and then estimated by the Folin-Denis method (AOAC 1984). Protein-precipitable

phenols were estimated by using a modified procedure of Hagerman and Butler (1978) as described by Jambunathan et al (1986). The vanillin assay was carried out with glacial acetic acid (Butler et al. 1982). Leucoanthocyanidin and proanthocyanidin were estimated according to Walterson and Butler (1983). All the results are expressed as absorbance units g^{-1} of defatted seed coat except in the case of the Folin-Denis method where the results are expressed as mg g^{-1} of tannic acid equivalents.

Results and Discussion

The ranges and means of seed colonization and polyphenols contents in 50 genotypes are shown in Table 1. A significant and negative correlation ($r = 0.33$, $P < 0.05$) was found between seed colonization by *A. flavus* and polyphenols content estimated by the Folin-Denis method. However, when the groundnut genotype OG 43-4-1, which showed 97% seed colonization and had the lowest polyphenols content (2 mg g^{-1}), was excluded from the analysis, the correlation coefficient ($r = 0.06$) became insignificant.

Table 1. Seed colonization (%) by *Aspergillus flavus* and polyphenol content in groundnut genotypes[1].

Number of genotypes		Seed colonization (%)	Polyphenols[2], ($\mu g\ g^{-1}$)	Correlation coefficient (r)
50	Range	25.6-97.0	2-388	-0.33*
	Mean	40.4	267	
	SE	±1.7	±10.1	
49	Range	25.6-71.6	149-388	0.06
	Mean	39.2	272	
	SE	±1.3	±8.6	

1. Rainy season 1982.
2. Estimated by the Folin-Denis method on acidified methanol extract.
* Significant at $P < 0.05$.

The reactions of 13 groundnut genotypes to in vitro seed colonization by *A. flavus* are shown in Table 2. All the resistant lines had less than 15% seed colonized, except ICGS 58 at Tirupati (19.8%) and ICGS 78 (39.3%), ICGS 58 (36.8%), and PI 337394 F (15.8%) at Anantapur. All the five susceptible genotypes had more than 30% of their seed colonized across all three locations. The ranges and mean values of polyphenols estimated by the Folin-Denis method in the acidified methanol extract of seed coats and by Prussian blue method in the methanol extract are shown in Table 3. Variation between resistant and susceptible cultivars at any of the locations was not apparent from these values. The vanillin assay method has been used as one of the standard methods for the estimation of degree of polymerization of proanthocyanidin. The ranges and mean values obtained did not show a large variation between the resistant and susceptible genotypes at any location though the absorbance values g^{-1} of the seed coat were high for both the resistant and susceptible genotypes (Table 4). In contrast, protein-precipitable