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Various Groundnut Seed Coats on Break Down of Genotypic Resistance to *Aspergillus flavus*

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Abstract

Experiments were set up to investigate the relationship between the invasion of this fungus and the differences of seed coat structure from 6 varieties of groundnut which have difference degree resistance to *Aspergillus. flavus*. Seed coat was measured and tested for resistance under both inoculated and non innoculated conditions, then seed were kept in 2 layers sealed plastic bags with the seed moisture content lower than 10 percent and kept under room temperature. Each month after storage, seed were sampled and tested for resistance to the *A. flavus*, viability using the dehydrogenase enzyme determination and total carbohydrate. It was found that the thickness of seed coat has no correlation with the resistance to *A. flavus*. However, cross-sectioned photographs from seed coat during a various period of time after inoculated the fungus showed that the resistance genotypes (CMU collection 1 and J 11) have shown an unchanged seed coat structure which in contrast, the susceptible genotypes (RCM 387 and Tainan 9) shown an breakdown in its seed coat structure. This phenomenon indicated that the seed coat of the resistance genotypes may compose of some chemical or tissues which could block the pathway and inhibit the spreading of the fungus's toxicity. In another word, its seed coat could characterize a cellular defense structure. Seed moisture content which lower than 10 percent stored for a period of 6 months were reducing infection of the fungus in all tested genotypes. Further more, it was observed that the genotypic breakdown genotypes (RCM 387 and Tainan 9) have markedly higher carbohydrate content than the non-genotypic breakdown one. Conclusion could therefore, be made that chemical and other structure of the seed coat except the seed thickness may play the important roles of this resistance. Also, the storage condition is another important external factor.

Keywords: seed coat, *Aspergilus flavus*, aflatoxin, peanut

Introduction

Aspergillus flavus causes mold of peanuts (*Arachis hypogaea* L.) and also produces aflatoxin as a result of contamination of this fungi (Diner and Davis, 1977). A study on resistance to *A. flavus* in peanut was done after a short period of storage by Karladee and Yingthongchai (1994). *A. flavus* infection was found only in laboratory test, but not in the field experiment. Genetic resistance in seed did nor show the protective effect of healthy seeds from *A. flavus,* which is called "genetic breakdown" the probable cause might be due to seed structure thereby seed coat or testae which prevents the cotyledons. Moreover, the accumulated food in the seed, *A. flavus* uses for their growth.

The increasing genetic resistance specially for seed coat against *A. flavus* and aflatoxin accumulation to improve seed health quality by breeding technique was done by breeding transform resistant gene to high yielding peanut varieties (ICRISAT, 1977). Further more, Mcdonald (1989) studied about combining genetic resistance of seed coat and aflatoxin accumulation at low level. These are the benefits of seed coat resistance in plant breeding. There are many new genetic resources that resistant against *A.flavus* in peanut seed. If the property and genetic breakdown of seed coat were correlated. It would be great benefit for improving healthy and good seed production. This experiment was conducted to study the morphology of peanut seed coat, which protects the cotyledon from *A. flavus* and also the relation between thickness and degree of resistance of various peanut genotypes. Moreover, seed sampling and testing for resistance against *A. flavus* was done including viability test and estimation of total carbohydrate content in peanut seed monthly after storage.

Material and method

Seeds of 6 varieties (CMU collection 1, (J11xRCM387)-8-6-2, CMU collection 3, J11, Tainan9 and RCM 387) into two parts. Half of them was used for study the inducing to fungus invasion by various structure of seed coat by freezing microtome method (Johansen, 1940) and the other was used for seed health laboratory test in monthly interval up to 6 months.

Experimental design : Analysis of variance was test by Completely Randomized Design (CRD) with 3 replications.

Freezing microtome method : The thickness of seed coat was determined by microscope and recorded in micrometer.

Laboratory Test : Seed were sampling monthly after storage and seed surface sterilized with 10% Sodium hypochlorite for 2 minutes followed by rinsing three times in distilled water. Surface sterilized seeds were inoculated with *A. flavus* spore suspension which were in advanced prepared. These inoculated seeds were used for the following purposes

a) Determine the degree of susceptibility: The inoculated seeds were incubated between paper. Data were recorded on the basis of percentage of *A. flavus* structure to determine the degree of susceptibility.

b) Viability test: The inoculated seeds were determined for viability testing dehydrogenase enzyme activities by tetrazolium method (ISTA, 1985). It was determined by the percentage of stained area on the whole cotyledon.

c) Effect of *A. flavus* on carbohydrate contents in seeds: In the inoculated seeds, carbohydrate content was analyzed following by Anthrone reagent method (Morris, 1948)

Mode of penetration of A. flavus to seed coat and its development **:** Seeds were sampling monthly and their surface were sterilized with 10% Sodium hypochorite. The surface sterilized seeds were inoculated with *A. flavus* spore suspension on the site of seed coat which were located earlier. Then incubated on moisture plate after the required time for conidial germination and the infected location were cut and separated. The infected pieces of seed

coat were kept in fixing solution following paraffin-section method (Sass, 1979). Then for determining the penetration of *A. flavus* on the seed coat, the scanning microscope was used and photographs were taken as well.

Statistical analysis: Data was analysed using a model specific for CRD using the ANOVA procedure of Steel and Torrie (1960)**.**

Results

The highest thickness of seed coat was found in CMU collection 1 variety (13.84 micron), which was closer to (J11xRCM387)-8-6-2(13.38 micron). The moderately thickness seed coat were found in CMU collection 3 and J11 variety (13.11 micron). And the lowest thickness seed coats were found in Tainan9 (12.64 micron) and RCM387 (12.55 micron). From Table 1, it shows that the degree of susceptibility of groundnut against *A. flavus* does not depend on the thickness of seed coat.

Table 1. The thickness of various peanut seed coats and the percentage of infested seed by *A. flavus*

Variety	Thickness of seed coat (micron)	Infested seed* (% /month)						
		0	1	2	3	4	5	6
CMU collection 1	13.84	0	0.67	2.67	10.00	14.67	79.33	100.0
(J11xRCM387)-8-6-2	13.38	0	0.67	6.00	18.67	34.67	47.33	100.0
CMU collection 3	13.11	0	2.67	6.00	20.67	26.67	66.67	92.0
J11	13.11	0	0	2.00	6.67	14.67	54.67	100.0
Tainan9	12.64	0	2.00	5.33	18.67	26.00	68.67	99.2
RCM 387	12.55	0	1.33	4.00	12.00	20.67	78.00	99.2
LSD(0.05)	0.692	-	ns	ns	8.26	8.87	13.05	1.11

*Remarkable: Degree of resistance - infested seed < 15% is resistance
- 15.01-30.00 % is moderately resistance
- 30.01-50.00 % is susceptible
- > 50.00 % is very susceptible(Mixon and Roger,1973).

In 3 months of storage, three varieties (CMU collection 3, (J11xRCM387)-8-6-2, Tainan9) showed moderate degree of resistance. But, After 4 months two varieties (J11, CMU collection 1) are showed almost resistance against *A. flavus*. After the 5^{th} and the 6^{th} month of storage, every variety showed the susceptibility to *A. flavus* infestation. Table 2 shows, in 3 months of storage, every variety still contains seed viability, but at the 4^{th} month, (J11xRCM387)-8-6-2 lost their viability (stained area less than 75 % of whole seed). Other varieties (CMU collection 1, CMU collection 3, J11) were still contained viability (stained

area are more than 75 % of whole seed). At last, in 6th month only J11 variety were able to contain seed viability. It might be due to protective mechanism by cellular defense structure.

Table 2. The carbohydrate contents and determined red area that located of dehydrogenase enzyme activity of infested seeds.

Variety	Carbohy-drate content(%)	Remark of stained area* (% /month)						
		0	1	2	3	4	5	6
CMU collection 1	29.63	1	1.15	1.30	1.53	1.76	2.19	2.10
(J11xRCM387)-8-6-2	31.26	1	1.09	1.16	1.75	2.18	2.24	2.52
CMU collection 3	36.33	1	1.18	1.19	1.26	1.76	2.22	2.18
J11	30.52	1	1.05	1.09	1.33	1.85	1.71	1.83
Tainan9	31.65	1	1.22	1.66	1.71	1.96	2.09	2.04
RCM 387	22.96	1	1.30	1.55	1.61	1.93	2.51	2.41
LSD(0.05)	0.692	-	ns	0.16	0.19	0.40	0.40	0.37

*Remarkable: stained area that located of dehydrogenase enzyme activity.
- 1 means stained area is 100 %of whole seed
- 2 means stained area is 75.00-99.99100 %of whole seed
- 3 means stained area is less than 75 %of whole seed

When determined the penetration of *A. flavus* by inoculation at seed coat it the sign of showed resistance especially at outer epidermis has which defenses itself. It is protection mechanism by cellular defense structure (Figure 1).

Conclusion

However, cross-sectioned photographs from seed coat during a various period of time after inoculated the fungus showed that the resistance genotypes (CMU collection 1 and J 11) have shown an unchanged seed coat structure which in contrast, the susceptible genotypes (RCM 387 and Tainan 9) shown an breakdown in their seed coat structure. This phenomenon indicated that the seed coat of the resistance genotypes may compose of some chemical or tissues which could block the pathway and inhibit the spreading of the fungus's toxicity. In another word, its seed coat could characterize a cellular defense structure. Seed moisture content which lower than 10 percent stored for a period of 6 months were reducing infection of the fungus in all tested genotypes. Further more, it was observed that the genotypic breakdown genotypes (RCM 387 and Tainan9) have markedly higher carbohydrate content than the non-genotypic breakdown one. Conclusion could therefore, be made that chemical and other structure of the seed coat except the seed thickness may play the important roles of this resistance. Also, the storage condition is another important external factor.

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