Chapter 4

Genotypic variation for resistance to *Aspergillus flavus* of groundnut germplasms

4.1 Introduction

Contamination of groundnut (*Arachis hypogaea* L.) by *Aspergillus flavus* and subsequent aflatoxins accumulation is a serious problem throughout the world, especially in subtropical and tropical regions. Pre-harvest *A. flavus* infection is one of the most consequences to produce aflatoxins in groundnut. Aspergillus colonization can occur during flowering or during aerial peg formation (Griffin and Garren, 1976) from viable air-borne propagules originating from groundnut soils (Griffin *et al.,* 2001; Horn *et al.,* 1995). The infection process is probably similar to infection of corn ears through the silks (Marsh and Payne, 1984). Flower and pegs can be contamination when temperatures are high during the morning (27-30 °C), but the infection occurs more often through the pegs after their penetration into the soil and during fruit, seed development and food and feed grains at the post-harvesting (Diener *et al.,* 1982; Sanders *et al.,* 1984).

The accumulation chemicals in plant cell have been found to inhibitory infection by pathogens. High calcium contents in cell wall decreased potato tubers infection by *Erwinia carotovora* subsp. *atroseptica* (Pagel and Heitefuss, 1989) and rice leaves invasion by *Helminthosporium oryzae* (Mukherjee and Ghosh, 1972). Copper and magnesium concentration were higher in resistant cucumber varieties than the susceptible to cucumber mosaic virus (Singh *et al.*, 1998). Grayer *et al.* (1992) and

Azaizeh and Pettit (1987) found that condensed tannin levels in groundnut have ability to against *Aphis craccivoro* and fungal attack. Thus, it is interesting to investigate whether groundnut peg and seed are resistant to *A. flavus* infection. Considering the protection role of certain chemicals and nutrients in cell wall development, it is hypothesized that higher chemical or nutritional content will result in stronger peg or seed and eventually less fungal infection. In pursuit of this aim we have studied the chemicals in groundnuts seedcoat response to *A. flavus* contamination.

Concerns over toxic and carcinogenic effects of aflatoxins in groundnut food products have stimulated much effort to reduce *A. flavus* infection. Genetic resistance to infection by aflatoxin producing fungi is a goal of groundnut breeder but has proved difficult to achieve (McDonald, 1989). One approach is to elucidate the mechanisms by which the plant resists the fungi and to enhance these by breeding and selection. Therefore, the present investigation was undertaken with the following objectives:

- To identify groundnut genotypes for resistance to pre-harvest and post-harvest *A. flavus* contamination.
- To evaluate chemicals content in groundnut peg and seedcoat that may associate to resistant to *A. flavus* infection.

4.2 Materials and Methods

4.2.1 Source of groundnut genotypes

Forty-six groundnut genotypes (Table 4.1) are collected from 1) Khon Kaen Field Crops Research Center, Khon Kaen, Thailand 2) International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India 3) University of Georgia, USA and 4) Department of Agronomy, Faculty of Agriculture, Chiang Mai University, Thailand. All genotype were grown at Field Experimental of Department of Agronomy, Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand between June to September 2001. At harvesting, mature pods were hand picked, field dried for 5-7 days, hand shelled, decreasing seed moisture content to 9-10 % and kept in refrigerator until used.

4.2.2 Pre-harvest determination: Peg screening method and AFHS technique

All groundnut genotypes were planted in 5-liter pots soil. A complete randomized design with four replications was used. Each groundnut cultivar was sprayed with aflatoxin producing *A. flavus* conidial suspension (prepared earlier as section 3.2.1.2) as inoculation (inoculation) and sterilized distilled water as uninoculation conditions at peg stage. Pots of groundnut were kept in growth chamber at 30 °C under 98 ± 2 % relative humidity. The pegs were collected at seven days after inoculation (Chapter 3). Each replicated peg was surface sterilized by 3 % sodium hypochlorite for 5 minutes and then soaked three times with sterile deionized water. After that, the sterile pegs were placed on M3S1B medium. A half of peg under inoculation condition was also observed with the novel screening technique, aniline blue fluorescence and hematoxylin staining (AFHS) technique as described in Chapter 3.

Table 4.1 Groundnut genotypes reaction to colonization by *Aspergillus flavus* and obtainable source.

Entry	Genotype identity	Obtainable source	Colonization Category
1	J11	Khon Kaen	Resistant
2	VRR245	Khon Kaen	Resistant
3	Tainan9	Khon Kaen	Susceptible
4	Lumpang	Chiang Mai	?
5	Sukhothai 38	Chiang Mai	?
6	KK4	Khon Kaen	Susceptible
7	KK5	Khon Kaen	Susceptible
8	KK60-1	Khon Kaen	Susceptible
9	US120	Chiang Mai	?
10	RCM	Chiang Mai	?
11	PRO40-1	Khon Kaen	?
12	Puaow	Chiang Mai	?
13	MaeTang	Chiang Mai	?
14	Kasetsart1	Chiang Mai	?
15	KKU90403	Khon Kaen	?
16	ICGX990090	ICRISAT	Resistant
17	ICGX990091	ICRISAT	Resistant
18	ICGX990092	ICRISAT	Resistant
19	ICGX990093	ICRISAT	Resistant
20	ICGX990094	ICRISAT	Resistant
21	ACC232	Georgia University, USA	Resistant
22	ACC329	Georgia University, USA	Susceptible
23	ACC419	Georgia University, USA	Susceptible
24	ACC511	Georgia University, USA	Resistant
25	Georgia Green	Georgia University, USA	?
26	PI355982	Khon Kaen	?
27	ICGS110	Khon Kaen	?

Table 4.1 continue

Entry	Genotype identity	Obtainable source	Seed colonization Category
28	ICGV86325	Khon Kaen	?
29	ICGV91066	Khon Kaen	?
30	ICGV91229	Khon Kaen	?
31	CM40-14-2	Khon Kaen	?
32	CM40-21-3	Khon Kaen	?
33	CM40-29-2	Khon Kaen	?
34	KK4xNCAC	Khon Kaen	?
35	TN9xVRR245	Khon Kaen	?
36	TN9xMoket	Khon Kaen	?
37	KK4xU4-7-5	Khon Kaen	?
38	(KK4xJ11)-5	Khon Kaen	?
39	(KK4xUF71513)-1	Khon Kaen	?
40	(KK4xAh7223)-10	Khon Kaen	?
41	(KK60-1 x EC36892)-17	Khon Kaen	?
42	(China x EC36892)-18	Khon Kaen	?
43	(KK60-2 x EC36892)-18	Khon Kaen	?
44	[(MGS9 x Chico)-12-16-1 x	Khon Kaen	?
45	[KK60-3 x (Ah65 x NCAC17090)]-3-11-7	Khon Kaen	?
46	ICGX920035	Khon Kaen	?
47	Nan	Nan Province	?

4.2.3 Post-harvest determination: Seed screening method

Sound mature seeds of uniform size and shape with intact seedcoat that kept in refrigerator are selected. Four separated groups of 10 g seeds (9.0 to 10.0 % seed moisture) are placed in 250-ml beakers for the inoculation and uninoculation screening methods respectively. The seeds were soaked for 6-minute intervals 3 times in 100 ml of sterile deionized water with 0.005 % Tween 20 L^{-1}. After the third soaking, the seeds were inoculated with 1 ml spore suspension *A. flavus* strain prepared earlier as section 3.2.1.2 (inoculation) and 1 ml of sterile deionized water for uninoculation. Then, they were placed aseptically in sterile petri dishes where sterile water is added to adjust seed moisture to 20 % (seed weight basis). Later, the dishes were put over water in semi-rigid plastic boxes and incubated at 30 °C under 98 ± 2 % relative humidity. Seven days after incubation, each replicated seed was surface sterilized by 3 % sodium hypochlorite for 3 minutes and then soaked three times with sterile deionized water. After that, the sterile seeds were placed on M3S1B medium (Griffin and Garren, 1974). The percentage of seeds colonized by *A. flavus* was recorded after cultured five days. The seeds were considered as colonized when colony erupts through their seedcoat and hyphae fluoresce in the medium under UV light (Figure 4.2). The percent infections were rated according to rating scale developed by Mixon and Roger (1973).

4.2.4 Minerals analysis in groundnut peg and seedcoat

Perkin - Elmer (1982): Aerial and soil peg and seedcoat were dried, peeled, grounded and sifted through a 2-mm sieve. Three replications of each genotype sample (between 1 to 2 g each) were weighed before placing in porcelain crucibles,

covered and then burned overnight in an electric muffle furnace at 450 °C. After cooling, several drops of analytic grade nitric acid were added to the ash samples before putting back in electric muffle furnace at 450 °C for another 15 - 16 hours. Later, the samples were dissolved in 5 ml of 6 N analytical grad HCL and diluted to 25 ml using deionized water. Lanthanum chloride 0.2 % was added to the samples and to standard solutions in order to overcome interference from silicon, aluminium, phosphate, and sulphate as well as ionization interference in the acetylene flame. The calcium, copper and magnesium contents of duplicated solution were determined by flame atomic absorption spectrophotometer (AAS Model; Perkin – Elmer 3100, USA) with data converted to $mg.kg^{-1}$.

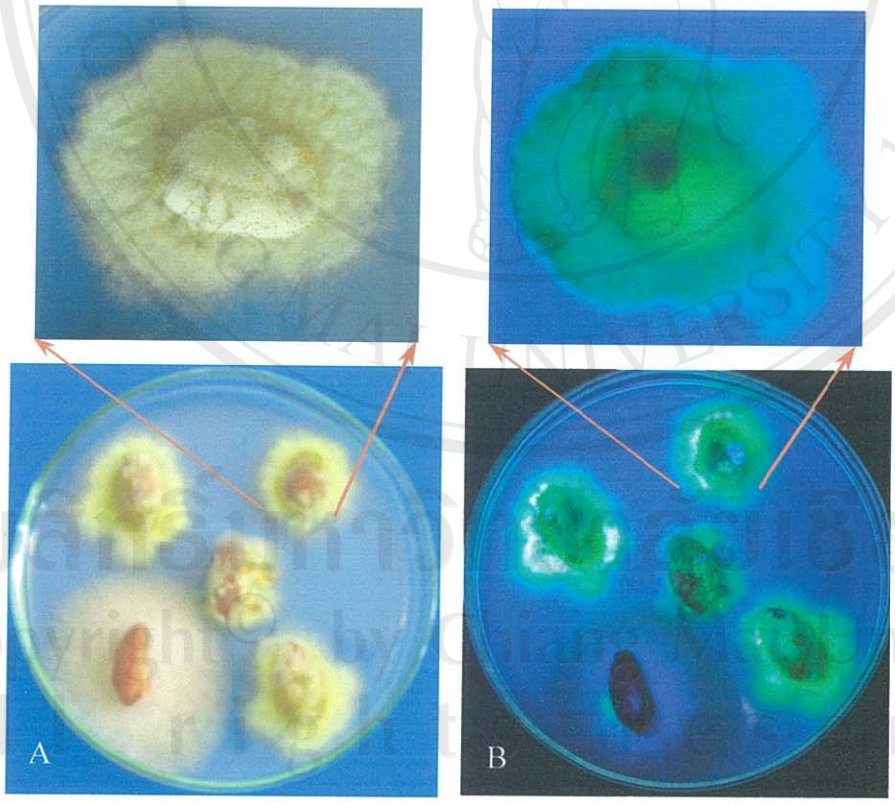

Figure 4.1 Inoculated seeds on M3S1B selective medium under normal light (A) and UV light (B).

4.2.5 Tannins analysis in groundnut peg and seedcoat

Makkar (1995): Soluble condensed tannins (CT) were extracted from finely grounded samples (200 mg) of each groundnut genotype in four replications with 10 ml of 70 % (v/v) aqueous acetone in Erlenmeyer flasks for 24 hours at the room temperature with occasional mixing by swirling. After extraction, the supernatant of each sample was decanted into a conical flask and mixed thoroughly. One ml aliquot of the supernatant from each sample was then added to 6 ml of 80 % (v/v) butanol-HCl reagent in duplicated test tubes. The tubes were capped and transferred to a pre-heated dry block heater at 100°C and maintained for 1 hour. They were then removed and cooled before decanting into vials and measured absorbance at wavelength of 550 nm using a single beam spectrophotometer (UV1601; Shimadzu, Japan).

For total bound CT analysis, the residue from the soluble tannins extraction was washed twice with 70% aqueous acetone and then freezes dried. Ten mg of each sample was then put into duplicated tubes with 6 ml of butanol-HCl reagent. The tubes were then placed into a 100°C pre-heated dry block heater for 1 hour. They were cooled and centrifuged at 3000x g for 10 minutes. The supernatant was then decanted into vials and its soluble CT was measured absorbance at same the wavelength of 550 nm. Blank samples containing the reagent were only included in measurements. Total tannins are the sum of soluble CT and total bound CT.

4.3 Results

4.3.1 Pre-harvest determination

Percent peg infection by *A. flavus* of groundnut genotypes differed significantly under inoculation conditions (Figure 4.2). However, in comparison to uninoculation, most genotypes under inoculation condition are not resisted with average percent-contaminated pegs of all genotypes being 46.80. The percentage of infected peg under uninoculation condition of all genotypes is 0.72 (Table 4.2). While, the percent peg infections under inoculation condition were between 4.1-95.0. Eight groundnut genotypes have percent infection lesser than 30.0 %, US120, J_{11}, 511 CC, PRO40-1, Lumpang, ICGX990093, ICGX990094 and (KK60-1 x EC36892)-17.

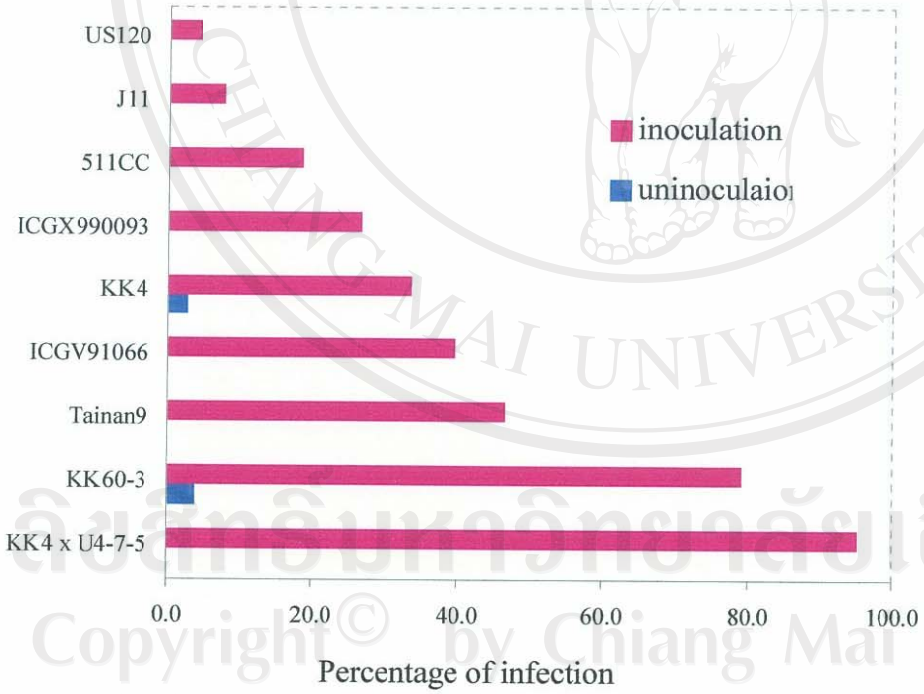

Figure 4.2 Percentages of groundnut peg contaminated by *Aspergillus flavus* under uninoculation and inoculation conditions.

Table 4.2 Percentages and resistant rate of groundnut pegs caused by *Aspergillus flavus* under uninoculation and inoculation conditions.

Genotypes, G	Uninoculation	Inoculation
	% Peg infection	% Peg infection
1. US120	0.00	4.10
2. J_{11}	0.00	7.40
3. 511CC	0.00	18.30
4. PRO40-1	0.00	21.40
5. Lumpang	0.00	25.00
6. ICGX990093	0.00	26.60
7. ICGX990094	0.00	27.10
8. (KK60-1 x EC36892)-17	0.00	29.90
9. Sukhothai38	1.40	30.60
10. Kasetsart1	3.60	30.70
11. ICGV91229	0.00	31.20
12. ICGS110	1.70	31.90
13. KK4	2.50	33.30
14. ICGX990091	0.00	33.30
15. PI355982	0.00	33.80
16. 419CC	0.00	34.80
17. KK5	0.00	35.00
18. CM40-14-2	1.70	36.30
19. ICGV91066	0.00	39.50
20. ICGX920035	0.00	41.10
21. (KK4 x UF71513)-1	5.00	41.20
22. Mae Tang	0.00	41.30
23. TN9 x VRR245	0.90	42.80
24. CM40-29-2	0.00	44.50
25. 232CC	0.00	44.80
26. KK60-1	0.00	45.00
27. Tainan9	0.00	46.30
28. Praw	0.00	46.80
29. (KK60-2 x EC36892)-18	0.00	47.60
30. ICGV86325	0.00	50.00

Table 4.2 continue

Genotypes, G	Uninoculation	Inoculation
	% Peg infection	% Peg infection
31. CM40-21-3	0.00	52.30
32. VRR245	0.00	53.90
33. (KK4 x Ah7223)-10	0.00	57.10
34. (KK4xJ11)-5	3.10	58.70
35. (China x EC36892)-18	3.60	63.60
36. ICGX990090	0.00	65.30
37. KK4xNCAC	6.30	66.70
38. 329CC	0.00	67.20
39. KKU90403	0.00	68.40
40. RCM	0.00	69.60
41. [(MGS9 x chico)-12-16-1 x KK60-3]	0.00	75.00
42. ICGX990092	0.00	75.30
43. KK60-3	3.50	78.90
44. TN9 x Moket	0.00	91.70
45.KK60-3x(Ah65xNCAC17090)]-3-11-7	0.00	92.30
46. KK4 x U4-7-5	0.00	95.00
Mean	0.72	46.80
Least significant difference, LSD (0.05)	**ns**	**27.01**

Percentages of peg area fluorescence by the infection of *A. flavus* of 43 groundnut genotypes were varied from 0.02 (J_{11} and ICGX990094) to 7.52 ((KK4 x UF71513)-1). There was highly significant difference among genotypes with the average of all genotypes as 2.16 %. While, after compared with the percent-infected pegs by peg screening method under inoculation condition found that the percentages of peg area fluorescence was not correlated to the percent-infected pegs at $p = 0.05$ (Table 4.3).

Table 4.3 Percentage of pegs infection and area fluorescence of peg tissue of 43 groundnut genotypes.

Genotypes	Percentage of pegs	
	Area fluorescence	Infection
J_{11}	0.02	7.40
ICGX990094	0.02	27.10
CM40-14-2	0.04	36.30
511CC	0.21	18.30
ICGX990093	0.26	26.60
CM40-21-3	0.32	52.30
232CC	0.48	44.80
KK4xAh-10	0.53	57.10
CM40-29-2	0.56	44.50
ICGV86325	0.88	50.00
KK60-3 x (Ah65xNCAC17090)]-3-11-7	1.11	92.30
PI355982	1.13	33.80
Lumpang	1.26	25.00
ICGX990091	1.39	33.30
Georgia green	1.40	NA
KK4xJ11	1.43	58.70
RCM	1.47	69.60
419CC	1.58	34.80
KK4xU	1.95	95.00
KK4xNCAC	1.99	66.70
[(MGS9 x chico)-12-16-1 x KK60-3]	2.02	75.00
Tainan9	2.03	46.30
SK38	2.04	30.60
ICGV91229	2.07	31.20
ICGX990090	2.15	65.30
ICGV91066	2.18	39.50
Maetang	2.19	41.30

Table 4.3 continue

Genotypes	Percentage of pegs	
	Area fluorescence	Infection
PRO40-1	2.36	21.40
ICGX990092	2.49	75.30
KKU90403	2.50	68.40
329CC	2.77	67.20
KK5	2.89	35.00
KK60-1	2.96	45.00
ICGS110	3.42	31.90
ICGV920035	3.47	41.10
KK60-1xEC-18	3.49	47.60
Kasetsart1	3.57	30.70
Phreaw	3.59	46.80
KK4	3.94	33.30
VRR245	4.18	53.90
KK60-1xEC-17	5.11	29.90
TN9xMoket	6.04	91.70
(KK4 x UF71513)-1	7.52	41.20
Mean	2.16	46.80
LSD (0.05)	3.01	27.01
Correlation coefficient at p=0.05	0.1707^{ns}	

Groundnut showed the significant differently of the conent of calcium, copper, magnesium, manganese, iron, zinc and tannins in pegs among genotypes (Table 4.4). The correlation coefficients of the chemicals contents to the percent-infected pegs under inoculation condition and percent-infected area fluorescence were analyzed. The significant relationships were found only in negative correlation of the percent-infected pegs to calcium contents in groundnut aerial pegs (r = -0.65**, n= 42) and soil pegs (r = -0.53*, n = 28) (Figure 4.3). The higher of the percent infected pegs of groundnut genotypes, the lower calcium concentrations in its peg structure.

Table 4. 4 Concentration of calcium (Ca), copper (Cu), magnesium (Mg), manganese (Mn), ion (Fe), zinc (Zn) and total tannins content in groundnut peg.

Genotypes	Ca (g/kg)		Cu (g/kg)		Mg (g/kg)		Mn (g/kg)		Fe (g/kg)		Zn (g/kg)		Total tannins
	Aerial	Soil	Aerial	Soil	Aerial	Soil	Aerial	Soil	Aerial	Soil	Aerial	Soil	(Δ550 / mg)
1	4.0	NA	0.1	NA	1.2	NA	0.8	NA	12.3	NA	0.6	NA	1.0
2	5.5	NA	0.4	NA	2.2	NA	1.4	NA	34.5	NA	2.4	NA	0.8
3	2.5	NA	0.1	NA	1.3	NA	0.9	NA	17.4	NA	1.2	NA	0.7
4	5.0	5.0	0.2	0.3	2.7	2.5	1.3	1.6	11.4	36.3	0.9	1.2	1.3
5	2.0	3.5	0.0	0.1	2.2	1.9	1.1	1.3	6.6	23.1	0.6	0.6	0.6
6	1.5	1.0	0.5	0.2	1.5	1.3	0.8	1.1	9.3	21.6	0.6	0.6	0.7
7	4.0	4.0	0.2	0.4	2.6	2.0	2.8	1.2	15.9	30.3	0.9	0.6	1.1
8	4.0	4.0	0.3	0.4	2.8	1.8	4.7	1.3	19.5	18.3	0.9	0.6	NA
9	4.7	5.0	NA	0.3	NA	1.9	NA	1.1	NA	20.4	NA	0.6	0.6
10	1.5	NA	0.1	NA	0.6	NA	0.9	NA	5.1	NA	0.3	NA	0.6
11	2.0	NA	0.1	NA	1.1	NA	0.7	NA	12.0	NA	0.3	NA	1.0
12	2.5	3.0	NA	0.4	NA	2.0	NA	1.2	NA	27.3	NA	0.6	1.2
13	3.0	3.0	0.1	0.2	2.8	1.9	1.2	1.1	9.3	23.1	0.6	0.6	0.5
14	2.5	3.5	0.1	0.2	0.9	1.9	0.7	1.5	NA	30.3	0.3	0.9	0.7
15	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.2
16	1.5	NA	0.2	NA	1.1	NA	0.8	NA	17.7	NA	0.6	NA	0.0
17	3.0	NA	0.2	NA	1.5	NA	1.4	NA	32.7	NA	1.5	NA	1.0
18	1.8	3.0	0.2	0.5	2.7	2.2	2.1	1.0	6.0	24.9	1.2	0.9	1.3

Table 4.4 continue

Genotypes	Ca (g/kg)		Cu (g/kg)		Mg (g/kg)		Mn (g/kg)		Fe (g/kg)		Zn (g/kg)		Total tannins (Δ550 / mg)
	Aerial	Soil	Aerial	Soil	Aerial	Soil	Aerial	Soil	Aerial	Soil	Aerial	Soil	
19	4.0	4.5	0.1	0.2	0.7	2.1	0.2	1.0	3.3	21.3	0.3	1.2	NA
20	4.5	4.0	0.1	0.2	2.4	2.2	2.1	1.1	13.8	29.7	0.9	0.6	0.8
21	1.5	3.0	0.1	0.2	0.9	1.5	0.9	1.5	7.5	29.4	0.6	0.6	1.2
22	2.0	2.0	NA	0.2	NA	2.7	NA	1.1	NA	24.6	NA	0.6	1.1
23	3.0	3.5	NA	0.2	NA	1.7	NA	1.3	NA	24.9	NA	0.6	NA
24	5.5	4.0	0.2	0.3	2.2	2.8	1.2	1.4	10.5	29.1	0.3	0.3	1.5
25	0.5	1.5	0.1	0.0	0.3	1.1	0.2	0.6	2.1	12.6	0.3	0.3	1.0
26	5.0	5.0	0.2	0.2	2.2	2.1	0.8	0.8	8.4	20.4	0.9	0.6	0.7
27	3.0	4.5	0.2	0.4	1.5	2.3	0.8	1.6	6.0	27.3	0.3	0.6	1.1
28	1.0	2.0	0.2	0.2	3.2	1.7	2.6	0.8	15.0	16.2	0.6	0.9	1.4
29	2.5	2.0	0.3	NA	1.8	NA	1.0	NA	20.1	NA	1.8	NA	1.1
30	4.0	NA	0.1	NA	2.1	NA	1.6	NA	37.5	NA	0.6	NA	NA
31	2.5	5.0	0.1	0.2	1.7	2.1	2.1	1.1	4.2	25.8	0.6	0.6	NA
32	2.0	NA	0.2	NA	1.6	NA	0.6	NA	24.6	NA	0.6	NA	0.9
33	2.5	3.0	NA	0.4	NA	0.9	NA	0.8	NA	17.4	NA	0.6	1.3
34	3.0	NA	0.2	NA	1.9	NA	1.2	NA	18.9	NA	NA	0.9	0.9
35	3.0	3.5	0.3	0.5	1.8	2.2	0.6	1.2	9.6	24.0	1.2	1.2	1.1
36	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.3

Table 4.4 continue

Genotypes	Ca (g/kg)		Cu (g/kg)		Mg (g/kg)		Mn (g/kg)		Fe (g/kg)		Zn (g/kg)		Total tannins (Δ550 / mg)
	Aerial	Soil	Aerial	Soil	Aerial	Soil	Aerial	Soil	Aerial	Soil	Aerial	Soil	
37	2.0	NA	0.1	NA	1.1	NA	0.6	NA	11.1	NA	NA	0.3	1.2
38	2.8	2.5	0.1	0.2	2.7	1.9	2.2	1.4	4.5	29.7	0.6	0.6	0.8
39	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.0
40	1.0	0.5	0.2	0.2	0.7	0.6	0.2	0.4	3.0	8.7	0.6	0.3	0.9
41	3.0	NA	0.1	NA	1.8	NA	1.2	NA	16.2	NA	NA	1.2	1.0
42	2.0	NA	0.3	NA	2.0	NA	1.0	NA	19.5	NA	0.9	NA	1.1
43	3.5	NA	0.2	NA	1.9	NA	1.5	NA	24.9	NA	0.6	NA	1.2
44	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.4
45	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.6
46	2.0	3.5	0.2	0.3	2.3	1.9	1.7	1.5	10.5	30.6	0.6	0.6	0.9
47	3.0	1.5	0.6	0.5	2.7	NA	2.5	1.6	9.3	25.5	0.9	0.9	0.3
Mean	**2.9**	**3.2**	**0.2**	**0.3**	**1.8**	**1.9**	**1.3**	**1.2**	**13.6**	**24.2**	**0.8**	**0.7**	**1.0**
SE	**1.2**	**1.3**	**0.1**	**0.1**	**0.7**	**0.5**	**0.9**	**0.3**	**8.8**	**6.1**	**0.5**	**0.3**	**0.3**
Correlation with percents-infected peg	**-0.65****	**-0.53***	-0.06	0.23	0.03	-0.43	0.21	-0.41	-0.45	-0.39	0.36	0.12	0.06
Correlation with percents-infected peg area	-0.28	-0.05	0.46	0.38	0.03	-0.04	0.03	0.26	-0.04	0.11	-0.03	0.12	-0.26

NA = not analysis

*,** Significant at p = 0.05 and 0.01 respectively.

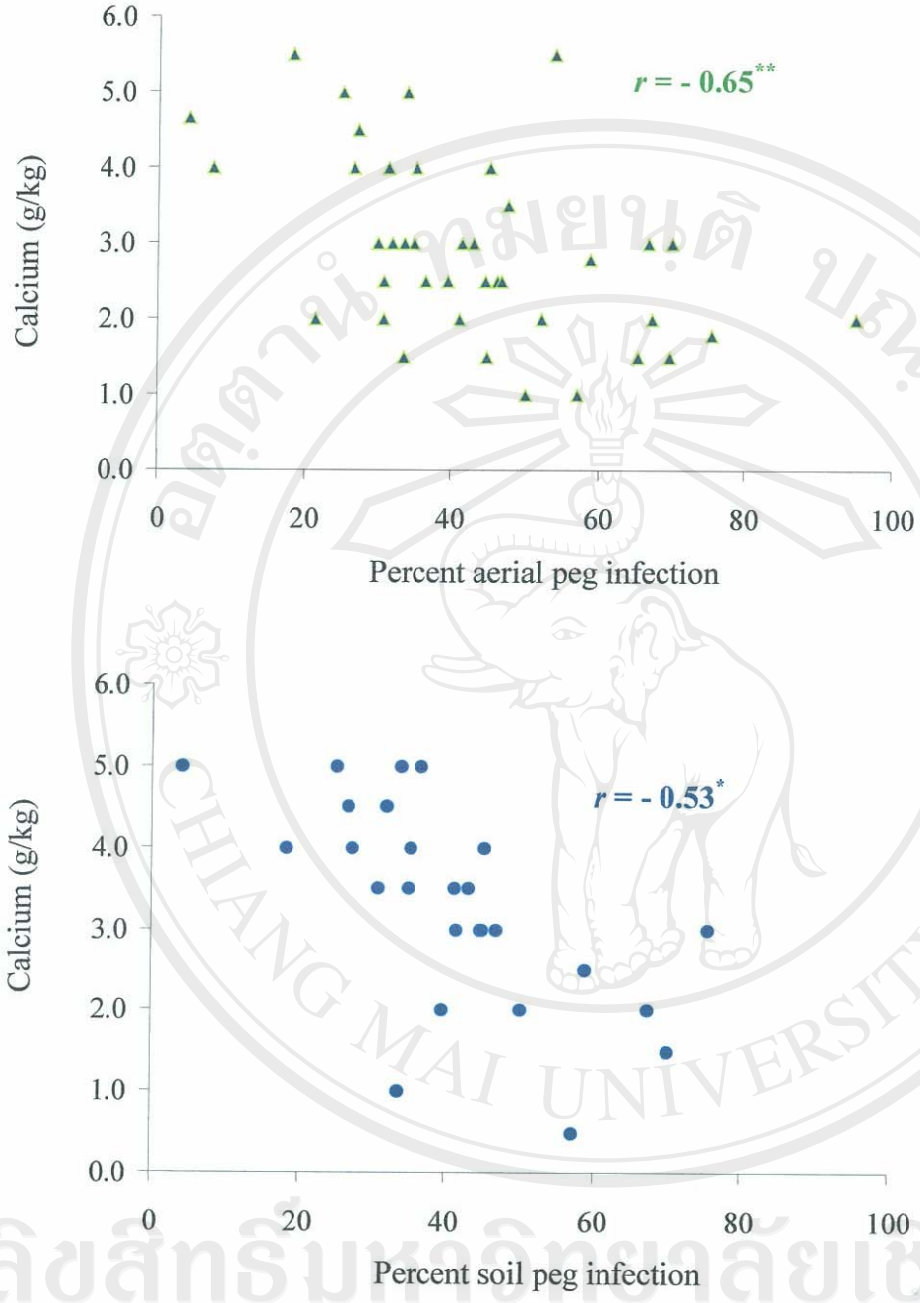

Figure 4.3 The correlation of percent peg infection with calcium (Ca) concentration in groundnut aerial pegs and soil pegs.

4.3.2 Post-harvest determination

Percent infection by *A. flavus* of groundnut genotypes differed significantly in both uninoculation (LSD = 8.1**) and inoculation (LSD = 19.5**) conditions. According to rating scale developed by Mixon and Roger (1973), 39 genotypes are classified as resistance (R), 5 genotypes as moderately resistance (MR) and 2 genotypes as susceptible (S) under uninoculation condition (Figure 4.4). However, in comparison to uninoculation, most genotypes under inoculation condition are susceptible and only 5 genotypes can be classified as MR. For the remaining of MR genotypes, 9 genotypes are classified as S and 32 genotypes as high susceptible (HS) with average percent-contaminated seeds of all genotypes is 62.90. The percentage of infected seed under uninoculation condition of all genotypes is 6.30 (Table 4.5).

When comparing resistant levels of each genotype under uninoculation and inoculation conditions, it is found that R or MR genotypes (39 genotypes) in uninoculation changed to S or HS in inoculation. For the remaining of 7 genotypes, five genotypes: US120, ICGX990093 ICGX990094, 511CC and 232CC are classified as R or MR. Other two genotypes: KK4 and ICGV91066 can be grouped as S or HS in both conditions (Table 4.5).

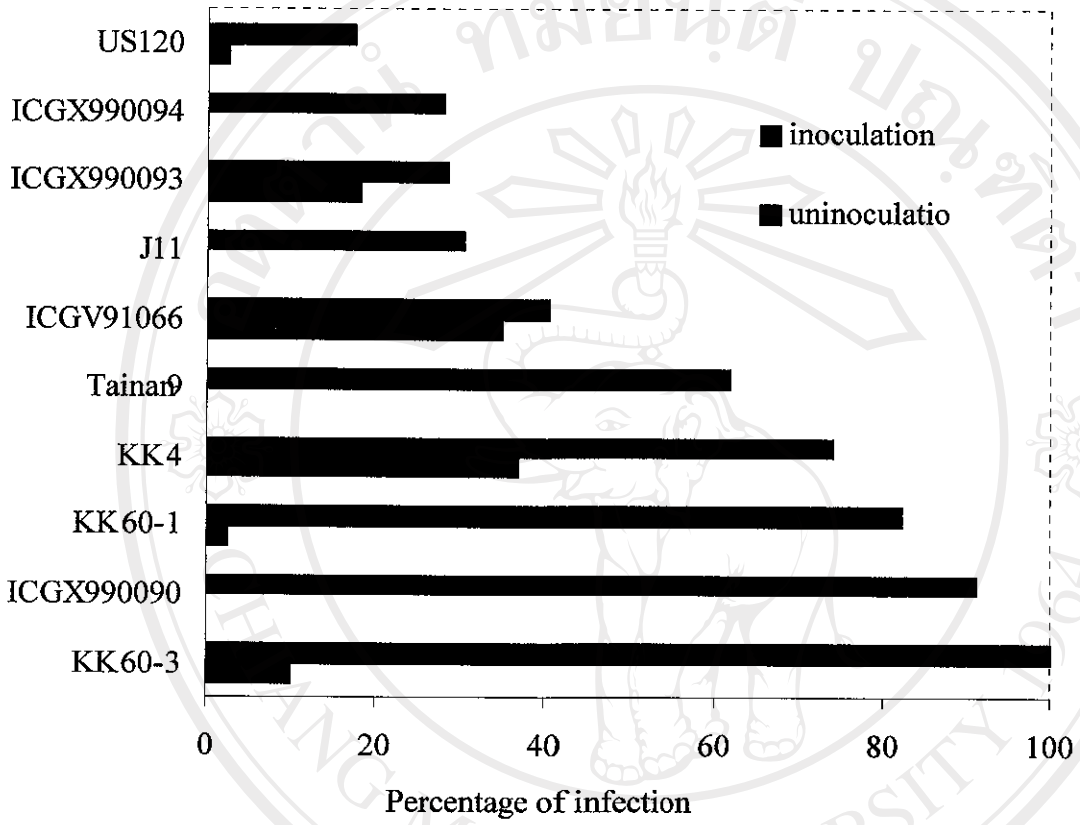

Figure 4.4 Percentages of groundnut seed contaminated by *Aspergillus flavus* under uninoculation and inoculation conditions.

Table 4.5 Percentages and reaction category of groundnut seed infected by *Aspergillus flavus* under uninoculation and inoculation conditions.

Genotypes, G	Uninoculation		Inoculation	
	Seed infection (%)	Reaction category *	Seed infection (%)	Reaction category *
1. US120	**2.5**	**R**	**17.5**	**MR**
2. ICGX990094	**0.0**	**R**	**27.9**	**MR**
3. 511CC	**5.0**	**R**	**28.6**	**MR**
4. ICGX990093	**18.1**	**MR**	**28.6**	**MR**
5. 232CC	**2.5**	**R**	**29.7**	**MR**
6. J_{11}	0.0	R	30.5	S
7. PI355982	25.0	MR	31.9	S
8. Mae Tang	5.8	R	32.6	S
9. Praw	7.2	R	33.9	S
10. TN9 x VRR245	0.0	R	37.5	S
11. KK4xNCAC	5.0	R	37.5	S
12. ICGX920035	2.5	R	37.8	S
13. ICGV91066	**35.0**	**S**	**40.5**	**S**
14. ICGV91229	0.0	R	42.5	S
15. VRR245	7.5	R	45.2	HS
16. (KK4 x Ah7223)-10	5.0	R	47.1	HS
17. KK4 x U4-7-5	7.5	R	53.3	HS
18. (KK60-2 x EC36892)-18	0.0	R	54.6	HS
19. TN9 x M0ket	2.5	R	60.6	HS
20. (KK60-1 x EC36892)-17	2.5	R	61.0	HS
21. [(MGS9 x Chico)-12-16-1 x KK60-3]	11.6	R	61.0	HS
22. Tainan9	0.0	R	61.5	HS
23.(Chico x EC36892)-18	0.0	R	63.0	HS
24. PRO40-1	0.0	R	64.4	HS
25. CM40-21-3	0.0	R	71.9	HS

Table 4.5 continue

Genotypes, G	Uninoculation		Inoculation	
	Seed infection (%)	Reaction category *	Seed infection (%)	Reaction category *
26. CM40-14-2	0.0	R	72.9	HS
27. KK4	**37.0**	**S**	**73.9**	**HS**
28. KK5	5.0	R	75.7	HS
29. (KK4 x UF71513)-1	17.5	MR	76.9	HS
30. Lumpang	17.5	MR	77.2	HS
31. KKU90403	2.5	R	78.3	HS
32. ICGX990091	0.0	R	79.6	HS
33. Kasetsart1	5.0	R	79.9	HS
34. Sukhothai38	5.0	R	80.8	HS
35. KK60-1	2.5	R	82.2	HS
36. ICGX990092	0.0	R	83.4	HS
37. ICGS110	0.0	R	86.0	HS
38. (KK4xJ11)-5	7.5	R	86.1	HS
39. ICGV86325	2.5	R	90.0	HS
40. ICGX990090	0.0	R	91.1	HS
41. 419CC	7.5	R	92.8	HS
42. RCM	0.0	R	92.9	HS
43. 329CC	16.6	MR	93.7	HS
44. KK60-3x(Ah65xNCAC17090)]-3-11-7	7.5	R	97.7	HS
45. CM40-29-2	0.0	R	100.0	HS
46. KK60-3	10.0	R	100.0	HS
Mean	6.30		62.90	
Least significant difference, LSD (0.01)	**8.1**		**19.5**	

Mixon and Roger (1973): % of *A. flavus* contamination between 0 - 15.00 = Resistance (R); 15.01-30.00 = Moderately resistance (MR); 30.01-50.00 = Susceptible (S); 50.01 - over = Highly susceptible (HS)

Genotypes also differed significantly in their contents of calcium, copper, magnesium and total tannins in the seedcoat (Table 4.6). The correlation between the percentage of seed infection under inoculation condition and the chemicals in seedcoat is shown in Figure 4.5. The only significant relationship to infection found is the negative correlation with concentration of calcium (r = - 0.5261**, n = 41) and tannins (r = - 0.4451**, n = 36). The lower the percentage of infected seed of groundnut genotype, the higher calcium and tannins content in their seedcoat. Five groundnut genotypes: US120, ICGX990093, ICGX990094, ACC232 and ACC511 can be classified as resistant, having infection rates between 0.0–29.4 % while genotypes KK4 and ICGV91066 showed susceptible to *A. flavus* infection under both conditions, having seed infection rates 35.0–74.0 % ($LSD_{Innoculation}$ = 25.77**, $LSD_{Uninoculation}$ = 10.72**). Calcium and tannins contents in the five resistant genotypes, US120, ICGX990093, ICGX990094, ACC232 and ACC511, were 5.90-6.44 $g.kg^{-1}$ and 1.447-1.810Δ_{550}. mg^{-1}, respectively. In the two susceptible genotypes, KK4 and ICGV91066, the calcium content varied from 5.06 to 5.26 $g.kg^{-1}$ and 0.98 - 1.126 Δ_{550}. mg^{-1} for that of tannins.

Table 4.6 Percent infected seed, calcium, copper, magnesium and total tannins content in seedcoat of groundnut genotypes.

Varieties	Infected seed (%)	Ca (g/kg)	Cu (g/kg)	Mg (g/kg)	Total tannins (Δ550 / mg)
1. US120	17.5	6.44	2.26	0.3551	1.537
2. ICGX990094	27.9	NA	NA	NA	NA
3. 511CC	28.6	NA	NA	NA	1.447
4. ICGX990093	28.6	NA	NA	NA	NA
5. 232CC	29.7	5.90	1.55	0.2397	1.81
6. J_{11}	30.5	6.08	3.08	0.2411	1.611
7. PI355982	31.9	6.39	1.36	0.2945	0.874
8. Mae Tang	32.6	6.39	2.89	0.2859	0.849
9. Praw	33.9	5.58	1.58	0.319	1.409
10. TN9 x VRR245	37.5	3.46	1.45	0.4547	1.129
11. KK4xNCAC	37.5	6.38	1.62	0.2671	1.203
12. ICGX920035	37.8	4.71	1.69	0.3378	1.355
13. ICGV91066	40.5	5.26	2.7	0.2714	0.980
14. ICGV91229	42.5	5.00	2.39	0.3638	0.801
15. VRR245	45.2	4.78	0.98	0.2512	1.029
16. (KK4 x Ah7223)-10	47.1	6.21	2.77	0.2902	NA
17. KK4 x U4-7-5	53.3	5.83	3.4	0.3219	NA
18. (KK60-2 x EC36892)-18	54.6	6.86	1.47	0.2325	1.178
19. TN9 x Moket	60.6	4.91	1.39	0.3046	1.291
20. (KK60-1 x EC36892)-17	61.1	6.40	1.49	0.2642	0.727
21. [(MGS9 x chico)-12-16-1 x KK60-3]	61.0	5.61	1.6	0.2931	NA
22. TN9	61.5	5.21	1.66	0.3003	1.023
23. (China x EC36892)-18	63.0	4.25	1.39	0.2988	1.161
24. PRO40-1	64.4	5.54	1.83	0.2988	NA
25. CM40-21-3	71.9	5.83	2.08	0.2613	1.109
26. CM40-14-2	72.9	5.14	1.7	0.2584	NA
27. KK4	73.9	5.06	1.63	0.2772	1.126

Table 4.6 continue

Varieties	Infected seed (%)	Ca (g/kg)	Cu (g/kg)	Mg (g/kg)	Total tannins (Δ550 / mg)
28. KK5	75.7	5.36	1.54	0.2873	1.175
29. (KK4 x UF71513)-1	76.9	5.96	1.44	0.3089	NA
30. Lumpang	77.2	5.39	1.35	0.2902	NA
31. KKU90403	78.3	4.62	1.22	0.2945	0.868
32. ICGX990091	79.6	5.07	1.82	0.2281	0.978
33. Kasetsart1	79.9	3.71	1.97	0.3234	1.090
34. Sukhothai38	80.8	4.89	2.28	0.4027	0.697
35. KK60-1	82.2	4.63	2.76	0.2671	1.149
36. ICGX990092	83.4	NA	NA	NA	0.741
37. ICGS110	86.0	4.19	1.47	0.2974	1.361
38. (KK4xJ11)-5	86.1	5.89	1.31	0.3162	1.185
39. ICGV86325	90.0	5.45	2.21	0.2988	NA
40. ICGX990090	91.1	4.88	2.27	0.2988	0.875
41. 419CC	92.8	NA	NA	NA	NA
42. RCM	92.9	3.98	3.03	0.3537	0.728
43. 329CC	93.7	4.47	1.77	0.2137	0.786
44. KK60-3x(Ah65xNCAC17090)]-3-11-7	97.7	4.61	2.63	0.2354	1.103
45. CM40-29-2	100.0	3.72	1.21	0.3205	1.362
46. KK60-3	100.0	4.11	3.06	0.1646	0.842
Least significant difference, LSD (0.05)		0.2433	0.3249	0.0409	0.1754
Correlation with percents of infected seed		-0.5261**	0.0001 ns	-0.2049 ns	-0.4451**
Test significant of r - value		Ca, Cu, Mg			Total tannins
		(0.05) = 0.3080			(0.05) = 0.3346
		(0.01) = 0.3846			(0.01) = 0.4304

NA = not analysis

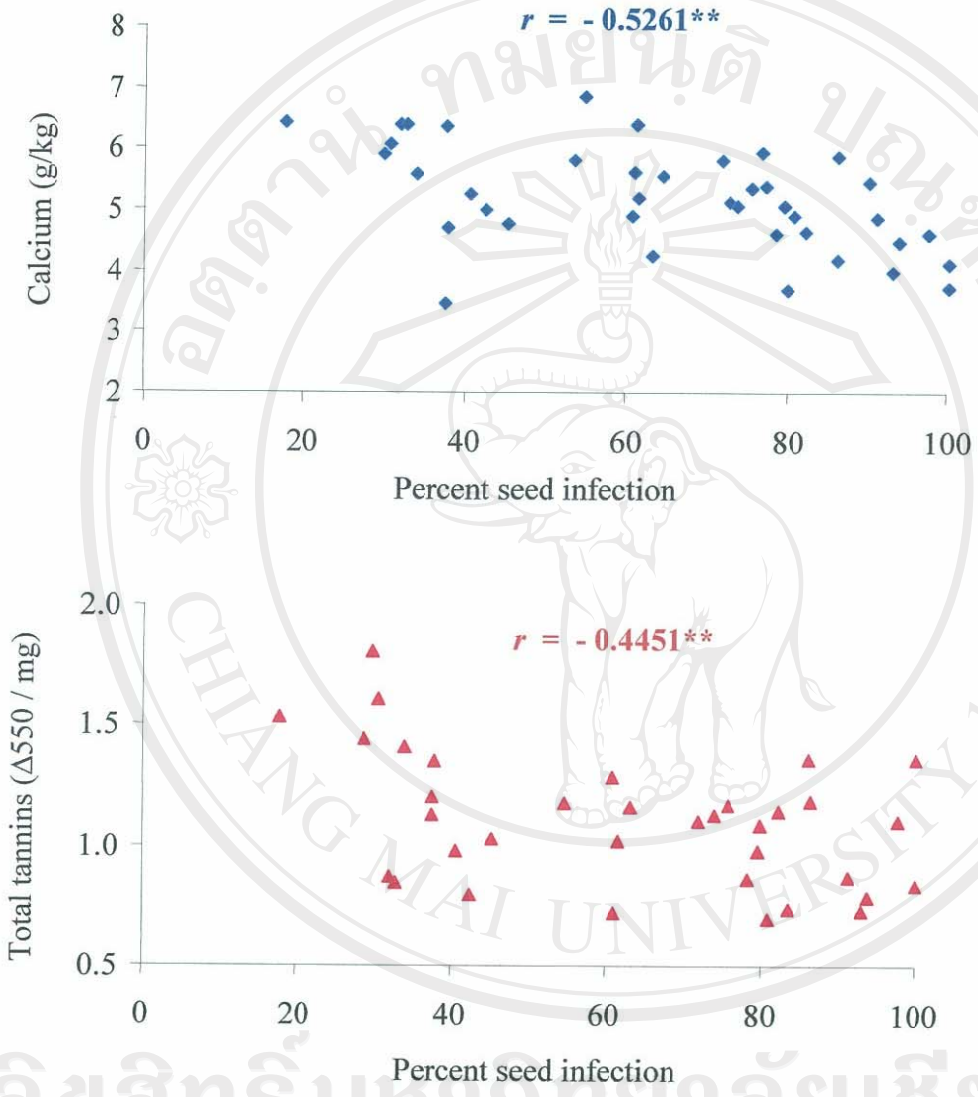

Figure 4.5 The correlation of calcium (Ca) and total tannins (Tannins) concentration of groundnut seed coat with percent seed infection.

4.4 Discussion

The results *A. flavus* contamination aerial pegs and seed of groundnut under uninoculation condition revealed that the fungus spread in the air around the field during groundnut growth and has ability to invasion during growing periods of groundnut. Due to Griffin *et al.* (2001), Horn *et al.* (1995) and Shearer *et al.* (1992) found the propagules of *A. flavus/parasiticus* in the soil of corn and groundnut fields and spore or conidia can spread in the air at the beginning of the growing season. While the difference among genotypes were found under inoculation condition that might be the suitable condition to screening the aerial peg and seed resistance to infection by *A. flavus*. According to Huang (2001), the disease incidences when constitutes the susceptible host, virulent and aggressive pathogens and optimum environmental. Likewise the condition for screening the plant disease resistant genotypes have to consist of the enough virulent pathogen to cause the disease and optimum environmental (Allard, 1966a), high moisture and temperature environments (Diener *et al.*, 1987; Sautour *et al.*, 2002).

Not significant correlation between the percent-infected peg and percent-infected peg area fluorescence may be caused of the difference of the quantitative character from peg screening method and qualitative value from AFHS technique. Although, the results represented that theirs chemicals concentration were not correlation to percent-infected peg area fluorescence. Thus, the difference among genotypes using AFHS technique was also effective for evaluation the resistant levels of groundnut peg for the qualitative character of resistance. The frequency of epidermal hair or papillae and the differently rough surface of peg structures support to obstruct the pathogen penetration (Huang, 2001; Comménil *et al.*, 1997). In

addition, the other chemicals from this present experiment would be helpfulness. The role of the cuticle wax, proteins, enzymes, other mineral; boron as barrier to fungal invasion is supported by a direct correlation between disease resistances in several host-parasite interactions (Chen *et al.,* 1998; Chen *et al.,* 1999; Huang *et al.,* 1997; Marschner, 1995). Therefore, the calcium in aerial pegs and soil peg were correlated to percent-infected pegs of groundnut. Calcium played important roles on cell division and elongation (Marschner, 1986) and as a constituent of the cell wall (Pagel and Heitefuss, 1989). Groundnuts pegs resisted to infection by *A. flavus* differently among genotypes in both peg screening method, AFHS technique and seed screening method.

From the present study, it can be concluded that the resistant genotypes expressed the low percent infection in both pre-harvest and post-harvest screening method, J_{11}, ICGX990093, ICGX990094, 511CC and the high percent infection indicated the susceptible genotypes, KK4 and ICGV19066. These genotypes could be used as potential sources of resistant and susceptible genes in a breeding program. In addition, calcium concentrations in groundnut peg and seedcoat and tannins in seedcoat appeared to inhibit *A. flavus* infection. Form the results could be suggested that calcium and tannins concentration might inhibit fungal growth or infection. Hence, calcium play important roles on cell division and elongation (Marschner, 1986) as well as being a constituent of the cell wall (Pagel and Heitefuss, 1989), which consequently controls thickness seedcoat of groundnut (Fernandez *et al.,* 1997). For the condensed tannins are polyphenolic compounds and accumulate in the plant cell with wide-range effects on microbes (Nicholson and Hammerschmidt. 1992). Azaizeh *et al.* (1990) and Lansden (1982) found that

some forms of methanol-extracted and water-soluble tannins extracted from seedcoat and cotyledons, when incorporated in yeast extract sucrose liquid medium, significantly inhibit *A. parasiticus* growth and reduce the level of aflatoxin produced.