CHAPTER IV

ASSOCIATIONS BETWEEN PHYSIOLOGICAL TRAITS FOR DROUGHT TOLERANCE AND AFLATOXIN CONTAMINATION IN PEANUT GENOTYPES UNDER TERMINAL DROUGHT

Introduction

Late season drought on peanuts (*Arachis hypogaea* L.) generally results in yield reduction, low seed quality, high incidences of *Aspergillus flavus* colonization, and high aflatoxin contamination. *Aspergillus flavus* colonization during the preharvest period is most important as it can serve as initial inoculum for further *A. flavus* colonization and, ultimately, aflatoxin contamination. Because aflatoxin is well recognized as a potent carcinogen, reduction of aflatoxin production is an important objective for peanut breeding programmes around the world.

Breeding progress for reduction of preharvest aflatoxin contamination (PAC) in peanut using field-based selection approaches have been slow due to large and uncontrollable genotype by environment (G x E) interactions (Anderson et al., 1995; Anderson et al., 1996; Holbrook et al., 1994). More consistent and simple traits with lower G x E interactions are worth exploring. A relationship between drought tolerance and reduced PAC has been demonstrated. Some drought-resistant genotypes (Rucker et al., 1995) were observed to have lower PAC when subjected to late season heat and drought stress (Holbrook et al., 2000a). From a breeding point of view, selection for drought tolerance could be an efficient strategy for reducing PAC.

This paper was accepted for publication in Plant Breeding (impact factor = 1.28), *doi: 10.1111/j.1439-0523.2009.01738.x*

Under drought stress, the loss of the capacity of peanut seeds to produce phytoalexins, an immune response to counteract fungal colonization resulted in higher PAC. The ability to maintain higher moisture contents in pods during drought periods may be an important trait enabling cultivars to resist aflatoxin production (Dorner et al., 1989; Wotton and Strange, 1985). In addition, drought resistance traits are promising as indirect selection tools for improving resistance to PAC in peanut. Researchers have shown a relationship of increased drought tolerance (canopy temperature, visual stress rating, and water use efficiency) and reduced aflatoxin production (Cole et al., 1993; Holbrook et al., 2000a; 2008; 2009).

The relationships between physiological traits related to drought resistance and PAC under terminal drought is not well understood. A better understanding might lead to the development of peanut cultivars with reduced PAC. The objectives of this study were to determine the effects of terminal drought on PAC and to investigate the associations between physiological traits for drought tolerance and PAC.

Materials and methods

Experimental design

The experiment was conducted under field conditions at Khon Kaen University located in Khon Kaen Province, Thailand during the dry season 2004/05, and repeated during the dry season 2005/06. A split-plot in a randomized complete block design with four replications was used. Main-plot treatments were two soil moisture levels [field capacity (FC) and 1/3 available soil water (1/3 AW) at 80 days after planting (DAP)] and sub-plot treatments were 11 peanut genotypes. Plot size was 2.5 x 3 m with spacing of 30 cm between rows and 10 cm between plants within a row. Rainout shelters were available if necessary.

Eleven peanut genotypes were used in this study. Eight (ICGV 98300, ICGV 98303, ICGV 98305, ICGV 98308, ICGV 98324, ICGV 98330, ICGV 98348, and ICGV 98353; medium maturing (110 days to maturity) and medium seeded type) are elite drought-resistant lines obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), one (Tifton-8; late maturing (120 days to maturity) and large seeded type) is a virginia-type line with a large root system

(Coffelt et al., 1985) received from the United States Department of Agriculture (USDA) and two are released cultivars ('KK 60-3'; late maturing (120 days to maturity) and large seeded type and 'Tainan 9'; early maturing (100 days to maturity) and medium seeded type) that are widely grown in Thailand. The lines from ICRISAT were identified as drought resistant because they gave high total biomass and pod yield in screening tests under drought-stress conditions (Nageswara Rao et al., 1992; Nigam et al., 2003; 2005). 'KK 60-3' is a virginia-type peanut cultivar having low pod yield under water stress, while 'Tainan 9' is a spanish-type peanut cultivar having low dry-matter production (Vorasoot et al., 2003).

Crop management

Soil was prepared by ploughing the field three times. Lime at the rate of 625 kg ha^{-1} was applied at first ploughing. Nitrogen fertilizer as urea at the rate of 31.1 kg N ha^{-1}, phosphorus fertilizer as triple superphosphate at the rate of 24.7 kg P ha^{-1} and potassium fertilizer as potassium chloride at the rate of 31.1 kg K ha^{-1} were incorporated into the soil by broadcasting during soil preparation prior to planting. Seeds were treated with captan (3a,4,7,7a-tetrahydro-2-[(trichloromethyl)thio]-1H isoindole- 1,3(2H)-dione) at the rate of 5 g kg^{-1} seeds before planting, and seeds of the two virginia-type peanut cultivars (KK 60-3 and Tifton-8) were treated with ethrel 48 % at the rate of 2 ml L^{-1} water to break dormancy. The seeds were over-planted and later the seedlings were thinned to obtain one plant per hill at 21 DAP. Weeds were controlled by the application of alachlor (2-cholro-2', 6'-diethyl-N-(methoxymethyl) acetanilide 48 %, w/v, emulsifiable concentrate) at the rate of 3 L ha^{-1} at planting and hand weeding during the remainder of the season. Gypsum ($CaSO_4$) at the rate of 312 kg ha^{-1} was applied at 47 DAP. Carbofuran (2,3- dihydro-2,2-dimethylbenzofuran-7-ylmethylcarbamate 3 % granular) was applied at the pod setting stage. Pests and diseases were controlled by weekly applications of carbosulfan [2-3-dihydro-2, 2-dimethylbenzofuran-7-yl (dibutylaminothio) methylcarbamate 20 % w/v, water soluble concentrate] at the rate of 2.5 L ha^{-1}, methomyl [S-methyl- N-((methylcarbamoyl)oxy) thioacetimidate 40 % soluble powder] at the rate of 1.0 kg ha^{-1} and carboxin [5, 6-dihydro- 2-methyl-1, 4-oxathine-3 carboxanilide 75 % wettable powder] at the rate of 1.68 kg ha^{-1}.

Water management

A subsurface drip-irrigation system (Super typhoon®; Netafim Irrigation Equipment & Drip Systems, Tel Aviv, Israel) with a distance of 20 cm between emitters was installed with a spacing of 30 cm between drip lines at 10 cm below the soil surface mid-way between peanut rows. Drip lines were fitted with a pressure valve and a water meter to ensure a uniform supply of the required water. Soil water level was maintained at FC at 0-60 cm depth. In the stress treatments, water was withheld at 60 DAP for 20 days according to 20 years historical pan evaporation data to allow soil moisture to gradually decline until reaching the predetermined levels of 1/3 AW at 80 DAP, and then the soil moistures were held fairly constant until harvest. Irrigation was applied regularly to prevent soil moisture from increasing or decreasing by more than 1 %. In maintaining the specified soil moisture levels, water was added to the respective plots by subsurface drip-irrigation based on crop water requirement and surface evaporation, which were calculated following the methods described by Doorenbos and Pruitt (1992) and Singh and Russell (1981), respectively.

Soil and weather data

Soil type was a Yasothon series (Yt: fine-loamy; siliceous, isohypothermic, Oxic Paleustults) with low available organic matter. Soil texture was sandy loam (sand 70.0 %, silt 22.5 %) with low clay content (7.5 %). Water holding capacity of the soil at FC and 1/3 AW was 12.90 % and 6.48 %, respectively. Soil moisture contents at 1/3 AW was the value between FC and permanent wilting point that was one third proportional to soil moisture at FC.

Soil moisture in each plot was monitored using the gravimetric method before planting, at planting, and three times after planting (60 DAP, 80 DAP, and at final harvesting) at the depth of 0-5, 25-30, 55-60 cm. Soil moisture volume fraction was also monitored at 10 days interval from planting to final harvest using neutron moisture meter (Type I.H. II SER, no. N0152, Ambe Didcot Instruments Co. Ltd, Abingdon, UK). Four aluminium access tubes were installed in each main-plot. Soil temperature was recorded by pocket thermometer (Checktemp 1, Hanna Inc, Woonsocket, Rhode Island, USA) between plants spacing at 10 cm depth at 60, 70, 80, 90, 100 DAP, and final harvest.

Relative humidity (%), pan evaporation (mm), rainfall (mm), maximum and minimum air temperature (^{o}C), and solar radiation (Cal cm^{-2}) during two cropping seasons were recorded daily from sowing until final harvest by a weather station located 50 m away from the experimental field.

A. flavus inoculation

Inoculum of toxigenic *A. flavus* was prepared and introduced into test plots to ensure the presence of sufficient aflatoxin-producing fungi in the peanut pod zone. The aflatoxin producing strain of *A. flavus* used in this study was isolated from peanut pods and cultured on selective Rose Bangal agar. Conidia of *A. flavus* from a 10 days culture were transferred to peanut-based medium (ground peanut seed and pods) and incubated at 25-30 ^{o}C for 14 days before being used as inoculum. The *A. flavus* inoculum at the rate of 375 kg ha^{-1} were broadcasted to peanut plots at 30 DAP.

Plant measurements and observations

Physiological traits were measured at 80 DAP. Relative water content (RWC) was measured following Kramer (1980), using the second fully expanded leaf from the top of the main stem. RWC was measured from five plants for each plot using one leaflet from each plant to evaluate plant water status around midday, 10–12 AM (Clavel et al., 2006). RWC at 60, 70, 100 DAP, and harvest were also measured to monitor the change in plant water status.

Data were recorded for SPAD Chlorophyll Meter Reading (SCMR) and Chlorophyll density (ChlD) to monitor the chlorophyll status. Five plants from each plot were randomly sampled, and the second fully expanded leaves from the top of the main stems were used for SCMR at 09–10 AM. SCMR was recorded using a Minolta SPAD-502 meter (Tokyo, Japan) on the four leaflets from each leaf (Nageswara Rao et al., 2001). ChlD were analyzed following the procedures described by Moran (1981).

Leaf area was measured from leaf sampling of five plants using a leaf area meter (LICOR-3100, LICOR Inc., Lincoln, Nebraska, USA) and average leaf area per plant was computed. Leaf dry weight from five plants was also measured after oven drying at least 48 hours at 80 ^{o}C and then specific leaf area (SLA) was computed.

Canopy temperatures were observed from each plot at 10-12 AM, using an infrared thermometer (TESTO 830 T1, Hotek Technologies, Tocoma, Washington, USA). Drought stress ratings (DSR) were also determined using the method of Del Rosario and Fajardo (1988).

Biomass and pod yield

Yield and total biomass in an area of 1.8 m^2 were harvested at maturity (R8) (Boote, 1982). A 1 kg random sample of shoots was oven-dried at 80 °C at least 48 h and dry weight was measured. Shoot dry weight per plot was then calculated. Pod yield was weighed after air drying to approximately 7–8 % moisture content. Drought tolerance indices (DTI) for each parameter were calculated for the trait under 1/3 AW to that under FC conditions as suggested by Nautiyal et al. (2002).

***A. flavus* and aflatoxin measurements**

Population of *A. flavus* in soil, *A. flavus* colonization, and aflatoxin contamination were measured following the methods described by Arunyanark et al. (2009). Soil samples were collected at 20, 40, 60, 80 DAP, and at final harvest to determine the population of *A. flavus*. One kg of soil was sampled from five positions in the pod zone for each plot and mixed well. Soil samples were placed in sterile plastic bags to provide a representative sample for each plot studied. The soil samples were immediately transported to the laboratory for microbial analysis. A sub-sample of 100 g was stirred with 125 ml of sterilized water for 1 min. The selective media was specifically designed for the isolation of organisms in the *A. flavus* group. The sample was diluted at 1:10 dilution factor and 100 µl was smeared on selective Rose Bangal agar in a glass petri plate. After incubation for 7 days at 25 °C, green colonies of *A. flavus* were counted. Colony forming unit (CFU) per 1 g of soil was computed by the following formula:

$$CFU / g\ soil = (A \times B \times C) / (D \times E)$$

Where, A is the number of *A. flavus* colonies, B is the volume of added sterilized water (ml), C is a dilution factor, D is the weight of the soil sub-sample (g), E is the volume of spreading soil solution (ml)

At harvest, pods from each plot were dried and hand shelled. One hundred

seeds were randomly selected to examine for *A. flavus* colonization. Seeds were surface sterilized by soaking in a 10% aqueous solution of Clorox (0.525 % NaOCl) for 5 min, rinsed with autoclaved distilled water, and placed on a moistened sterilized germination paper in a sterilized box. After 7 days incubation at room temperature (25-30 °C), seeds were examined for green conidial heads of *A. flavus* to determine the percent colonization. Aflatoxin contamination was determined by using final random 100 g seed sample from each plot. Aflatoxin B_1 was analyzed by a competitive Enzyme Linked Immunosorbent Assay (ELISA) method follow by Arunyanark et al. (2009).

Statistical analysis

Analysis of variance was performed for total biomass, yield and physiological traits in each year. Duncan's multiple range tests were used to compare means. All calculations were performed using the SAS PROC GLM version 6.12 (SAS Institute, 1990). Multiple-linear regression was used to determine the relative contribution of surrogate traits for drought resistance under stress conditions to *A. flavus* colonization and PAC. The analysis was based on the following statistical model (Gomez and Gomez, 1984):

$$Yi = \alpha + \beta 1X1i + \beta 2X2i + \delta i$$

where Yi is *A. flavus* or aflatoxin contamination under drought stress of genotype i, α is the Y intercept, X1i and X2i are surrogate traits (ex. SLA, SCMR and ChlD) of drought resistance under different water regimes of genotype i, respectively, $\beta 1$ and $\beta 2$ are regression coefficients for the independent variables X1 and X2, and δi is the associated deviation from regression. The analysis was carried out by fitting the full model first and then determining the relative importance of the individual independent variables. A sequential fit was then performed by fitting the more important variables first. The relative contributions of the individual independent variables to aflatoxin contamination under drought stress were determined from the percentages of regression sum of squares due to the respective independent variables to total sum of squares in the sequential fitted analysis. Simple correlations were used to determine the relationships between surrogate traits for drought resistance and seed colonization and PAC.

Results

Meteorological conditions

Relative humidity (%), pan evaporation (mm), rainfall (mm), maximum and minimum air temperature (^{o}C), and solar radiation (Cal cm^{-2}) during two cropping seasons were recorded. There was no rain during the 2004/2005 growing period (Figure 1). In the 2005/2006 season, most of the rainfall was received at the end of the season. Twenty three mm of the total amount of rainfall was recorded during 80-100 DAP. The difference in rainfall had no significant effects on parameters measured because all experimental plots were covered by rainout shelters. The differences between years in relative humidity and solar radiation were not significant. However, pan evaporation, rainfall, and maximum and minimum air temperature in the second year were higher than in the first year. Daily evaporation ranged from 2.2 to 8.3 mm in 2004/05 and 2.8 to 9.0 mm in 2005/06. The maximum and minimum air temperature ranged from 10.5 to 36.6 ^{o}C in 2004/05 and 12.0 to 38.7 ^{o}C in 2005/06, being lower during 60–110 DAP in 2004/05. Relative humidity ranged from 64 to 95 % in 2004/05 and from 51 to 96 % in 2005/06.The seasonal mean solar radiation was 0.12 Cal cm^{-2} in both years.

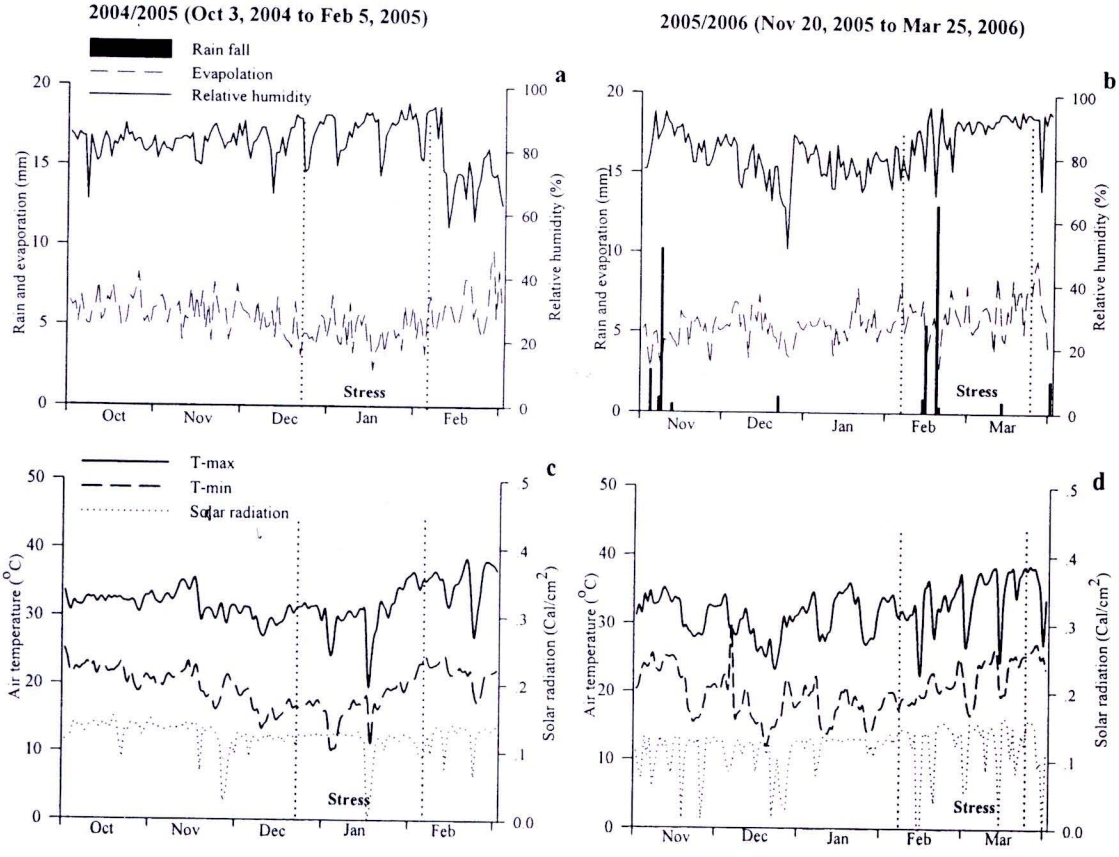

Figure 1 Relative humidity (%) (a and b), pan evaporation (mm) (a and b), rainfall (mm) (a and b), maximum and minimum air temperature (°C) (c and d), and solar radiation (Cal/cm^2) (c and d) during the crop growth period in 2004/2005 (Oct 3, 2004 to Feb 5, 2005) (a and c) and in 2005/2006 (Nov 20, 2005 to Mar 25, 2006) (c and d).

Soil data and plant water status

Soil temperatures between the two years were different. Averaged soil temperatures under water stress during growing years were 23.2 °C and 24.6 °C in 2004/05 and 2005/06, respectively (Figure 2). Soil temperatures under the drought at 80 DAP to harvest were clearly higher than the irrigated treatment. Soil temperatures in the stress treatment during the end of the season (80-120 DAP) were 23.4 to 24.7 °C in 2004/05, and 23.6 to 28.7 °C in 2005/06.

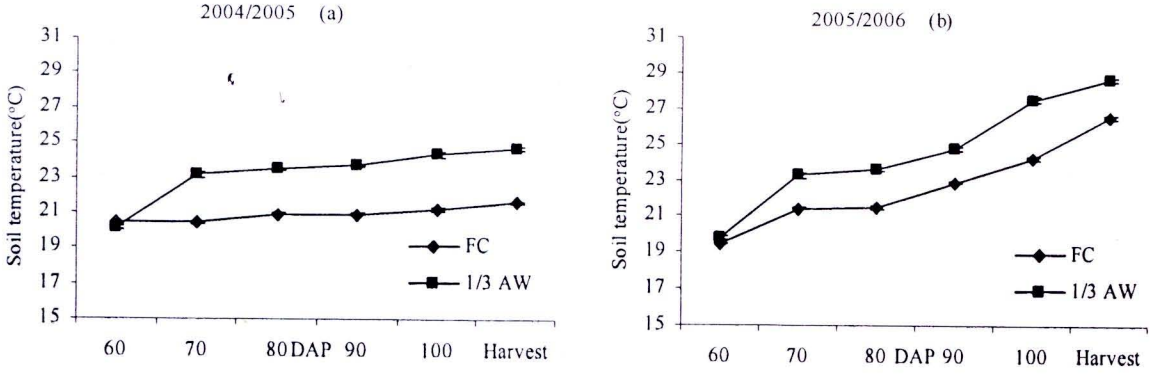

Figure 2 Seasonal changes in mean soil temperature (°C) at 60, 80, 90, 100 days after planting (DAP), and final harvest at 0-10 cm depth under difference water regimes [field capacity (FC) and 1/3 available water (1/3 AW)] in 2004/05 (a) and 2005/06 (b).

Soil moisture in the stress treatment at 80 DAP (6.4 % in 2004/05 and 6.9 % in 2005/06) was less than the non stress treatment (12.3 % in 2004/05 and 13.0 % in 2005/06) (Figure 3). After 80 DAP, soil moisture content of both treatments were held fairly constant until harvest. In general, soil temperature and soil moisture were well within the range likely to favor aflatoxin production.

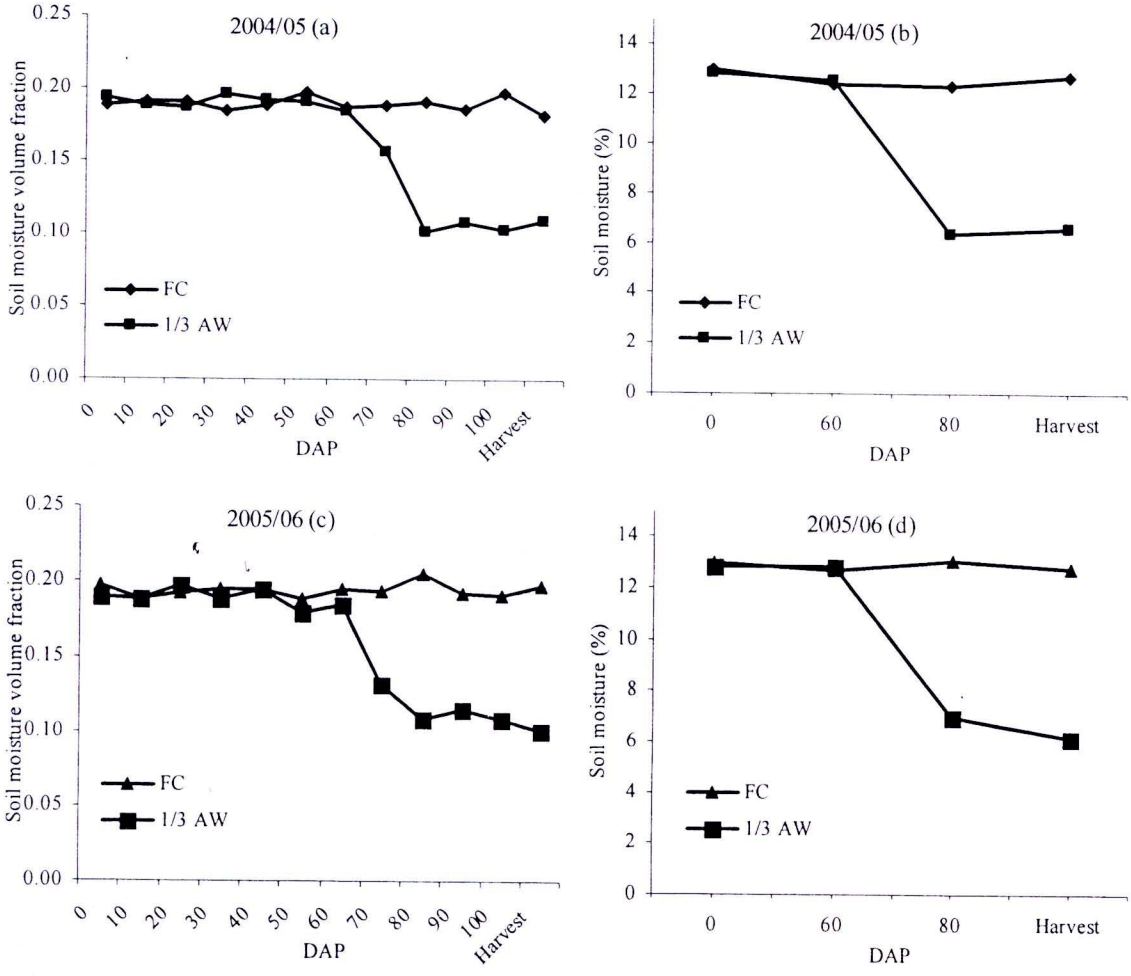

Figure 3 Soil moisture volume fraction (a and c) at planting, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 days after planting (DAP), and at final harvest and gravimetric soil moisture content (b and d) at planting, 60, 80 DAP and at final harvest under different water regimes [field capacity (FC) and 1/3 available water (1/3 AW)] average from 0-60 cm depth in 2004/2005 (a and b) and 2005/2006 (c and d).

The effect of drought on peanut could also be seen from the values of RWC of peanut under different water treatments (Figure 4). Relative water contents were >90 % at 60 DAP in both years. The effect of drought stress was evident with the decline in RWC to 80 % in the 1/3 AW treatments by 80 DAP. These results confirmed the soil moisture data in indicating that the degrees of drought stress were reasonably controlled at the predetermined levels.

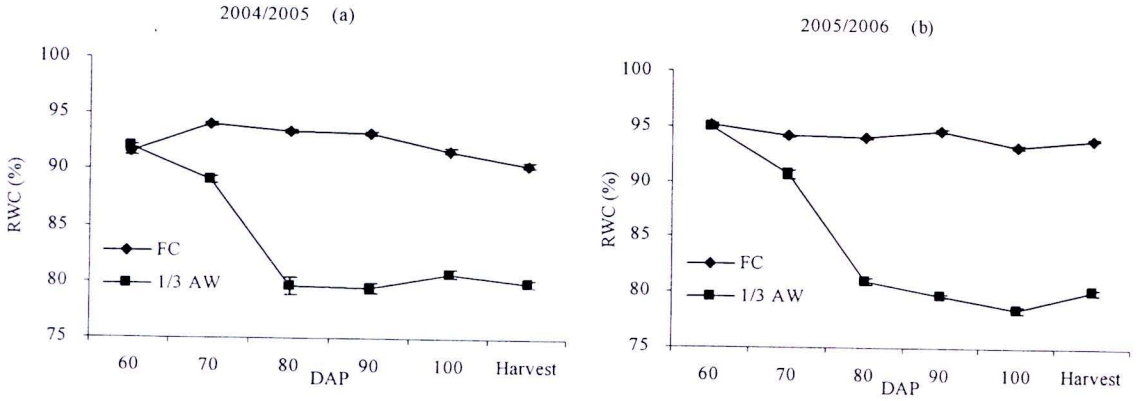

Figure 4 Leaf relative water content (RWC) of 11 peanut genotypes under different water regimes [field capacity (FC) and 1/3 available water (1/3 AW)] in 2004/05 (a) and 2005/06 (b).

Soil populations of *A. flavus* at 60 DAP to final harvest were significantly different between water regimes in both years (Figure 5). Drought soils had higher *A. flavus* populations than irrigated soils. Populations of *A. flavus* were highest at final harvest under both FC and 1/3 AW.

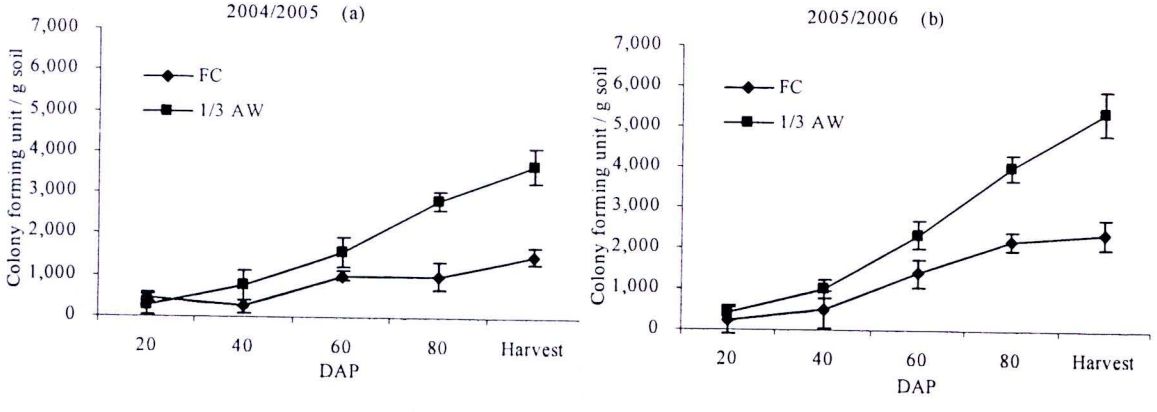

Figure 5 Soil populations of *A. flavus* at 60, 80, 90, 100 days after planting (DAP), and final harvest under different water regimes [field capacity (FC) and 1/3 available water (1/3 AW)] in 2004/05 (a) and 2005/06 (b).

Variability of total biomass and pod yield

Differences in years for total biomass and pod yield were found and G x E interaction effects for them were also significance. The results of this study demonstrated that terminal drought has more effect on pod yield than on biomass. Terminal drought reduced pod yield by 35 % and 34 % in 2004/05 and 2005/06, respectively, but only reduced biomass by 21 % in each year (Table 1). Under well-watered conditions, average biomass was 11072 and 10041 kg ha^{-1} in 2004/05 and 2005/06, respectively. Average pod yield under well-watered conditions was 4539 and 3977 kg ha^{-1} in 2004/05 and 2005/06, respectively. Under terminal drought, average biomass was 8755 and 7950 kg ha^{-1} in 2004/05 and 2005/06, respectively. Average pod yield under terminal drought was 2939 and 2634 kg ha^{-1} in 2004/05 and 2005/06, respectively.

According to G x E interaction effects, responses of peanut genotypes to terminal drought were different between years. Differences among peanut genotypes for total biomass and pod yield under different water regimes were found in both years. The results also revealed that variations in pod yield and the reductions in pod yield were relatively high compared to those for biomass production and the DTI of biomass. 'KK 60-3' and Tifton-8 were identified as genotypes with relative high total biomass and pod yield under well-watered and terminal drought conditions. In contrast, Vorasoot et al. (2003) found that 'KK 60-3' had low pod yield under drought compared with other genotypes, when their experiment was conducted under pot conditions. This may have resulted from a limitation of growing area in pots that limited pod production of virginia-peanut genotypes. However, the reductions in biomass and pod yield of these genotypes were relatively high especially for pod yield (low DTI). Genotype ICGV 98324 had high DTI for total biomass in both years. ICGV 98348 also had high DTI for pod yield across years. ICGV 98324 and ICGV 98348 were categorized as having medium biomass and pod yield under well-watered conditions but relatively low reductions in total biomass and pod yield (high DTI). 'Tainan 9' performed poorly for total biomass and pod yield under terminal drought, and had the highest reduction in total biomass. ICGV 98348 had remarkably high pod yield (3629 and 3390 kg ha^{-1} in 2004/05 and 2005/06, respectively) under water-stressed conditions. Drought-tolerant genotypes based on high DTI of total biomass

and pod yield were ICGV 98305, ICGV 98324, and ICGV 98348. Tifton-8 and 'KK 60-3' exhibited the highest biomass production under terminal drought in 2004/05 and 2005/06, respectively, but reductions in pod yield of both genotypes were also high.

Table 1 Total biomass (kg ha^{-1}) and pod yield (kg ha^{-1}) and drought tolerance index at harvest of 11 peanut genotypes grown under different water regimes in 2004/05 and 2005/06 dry seasons.

	Total biomass (kg ha^{-1}) in 2004/05			Total biomass (kg ha^{-1}) in 2005/06			Pod yield (kg ha^{-1}) in 2004/05			Pod yield (kg ha^{-1}) in 2005/06		
Genotypes	FC	1/3AW	DTI	FC	1/3AW	DTI	FC	1/3AW	DTI	FC	1/3AW	DTI
ICGV98300	12150ab	8837bcd	0.73	10286abc	7560cd	0.73	4420bc	2902bcd	0.66	3546b	2435bcd	0.69
ICGV98303	11226c-e	8620cd	0.77	10194abc	7668cd	0.75	4885abc	3318ab	0.68	4368ab	3007ab	0.69
ICGV98305	10516c-e	8727cd	0.83	9548abc	7862cd	0.82	4123c	2721bcd	0.66	3855ab	2775bcd	0.72
ICGV98308	11092c-e	8005cd	0.72	10406abc	7347cd	0.71	4160c	2830bcd	0.68	4308ab	2334cd	0.54
ICGV98324	10013cde	9103bc	0.91	9222bc	8475abc	0.92	3930c	3052abc	0.78	3585b	2348cd	0.66
ICGV98330	11091c-e	8849bcd	0.80	9250bc	7235cd	0.78	4754abc	2817bcd	0.59	3641ab	2396bcd	0.66
ICGV98348	11313bcd	9048bc	0.80	8996c	8117bcd	0.90	4900abc	3629a	0.74	3958ab	3390a	0.86
ICGV98353	9399e	7847d	0.83	9973abc	7740cd	0.78	4035c	2558cd	0.63	4603a	2555bcd	0.56
Tainan9	9606de	6528e	0.68	9662abc	6797d	0.70	3937c	2263d	0.57	3691ab	2512bcd	0.68
KK60-3	11775bc	9860b	0.84	11597a	9398a	0.81	5208ab	3152abc	0.61	4378ab	2938abc	0.67
Tifton-8	13611a	10877a	0.80	11321ab	9249ab	0.82	5582a	3087abc	0.55	3814ab	2289d	0.60
Mean	11072	8755	0.79	10041	7950	0.79	4539	2939	0.65	3977	2634	0.66
C.V. (%)	10.3	7.9		13.7	10.1		13.5	14.7		14.6	14.4	

Different letters in each column are significant at 0.01 level of probability by Duncan's multiple range test. FC, field capacity; AW, available soil water; DTI, drought tolerant index. DTI for a genotype were calculated by the ratio of stress/non-stress conditions.

Effects of drought stress on *Aspergillus flavus* colonization and aflatoxin contamination

Seed colonization by *A. flavus* and preharvest aflatoxin accumulation were increased when peanut were exposed to terminal drought (Table 2). Differences among peanut genotypes for seed colonization and PAC under well-watered conditions were not found in either year. Variability in *A. flavus* colonization and PAC under drought was found among the 11 genotypes evaluated. Variations in seed colonization and PAC under drought were also higher than those under fully irrigated conditions. Under terminal drought, seed colonization by *A. flavus* ranged from 11 to 20 % in 2004/05 and from 12 to 22 % in 2005/06. The average seed colonization increased from 2 to 15 % and from 2 to 17 % in 2004/05 and 2005/06, respectively. Mean aflatoxin contamination also increased from 2 to 332 ppb and from 1 to 817 ppb in 2004/05 and 2005/06, respectively. Aflatoxin contamination under drought ranged from 198 to 480 ppb in 2004/05 and from 535 to 1080 ppb in 2005/06.

ICGV 98308 had the highest seed colonization (20 and 22 % in 2004 and 2005, respectively). Tifton-8 exhibited the lowest seed colonization by *A. flavus* (11 and 12 % in 2004 and 2005, respectively). ICGV 98305, ICGV 98348, ICGV 98353, ICGV 98330, and Tifton-8 seemed to have consistently low aflatoxin contamination across years (Figure 6). The highest PAC was found in ICGV 98300, ICGV 98308, 'Tainan 9', and 'KK 60-3'. This study demonstrated that some drought resistant genotypes had lower *A. flavus* colonization and PAC.

Table 2 *A. flavus* colonization and aflatoxin contamination of 11 peanut genotypes grown under different water regimes in 2004/05 and 2005/06 dry seasons.

Genotypes	*A. flavus* (%)in 2004/05		*A. flavus* (%) in 2005/06		Aflatoxin (ppb) in 2004/05		Aflatoxin (ppb) in 2005/06	
	FC	1/3AW	FC	1/3AW	FC	1/3AW	FC	1/3AW
ICGV98300	2	18ab	2	19ab	2	480a	2	967abc
ICGV98303	2	16abc	3	17abc	1	345bcd	2	864a-d
ICGV98305	2	15abc	2	19ab	1	299cde	2	739b-e
ICGV98308	2	20a	2	22a	1	438ab	1	965abc
ICGV98324	2	14abc	2	17abc	2	299cde	1	803a-e
ICGV98330	2	15abc	3	16bc	1	281cde	0	652cde
ICGV98348	2	16abc	2	15bc	1	198e	1	535e
ICGV98353	2	14abc	2	15bc	2	259de	1	558de
Tainan9	2	17ab	2	18abc	2	440ab	2	1006ab
KK60-3	1	14bc	2	15bc	2	403abc	2	1080a
Tifton-8	1	11c	2	12c	1	209e	1	820a-e
Mean	2	15	2	17	2	332	1	817
C.V. (%)	32.5	20.8	22.9	20.3	38.4	24.7	28.9	24.4

Different letters in each column are significant at 0.01 level of probability by Duncan's multiple range test. FC, field capacity; AW, available soil water.

Seasonal effects on aflatoxin contamination and *A. flavus* colonization

Close associations were observed for seed colonization and PAC between the two years under both water regimes (ranged from r = 0.63* to r = 0.78**) (Figure 6). Correlation coefficients for *A. flavus* colonization between the two years were 0.78 ($P \leq 0.01$) and 0.68 ($P \leq 0.01$) under well-watered conditions (Figure 6a) and terminal drought (Figure 6c), respectively. Correlations for PAC between the two years was stronger under drought (correlation coefficient increased from 0.63 ($P \leq 0.05$) to 0.71 ($P \leq 0.01$)) (Figure 6b and 6d).

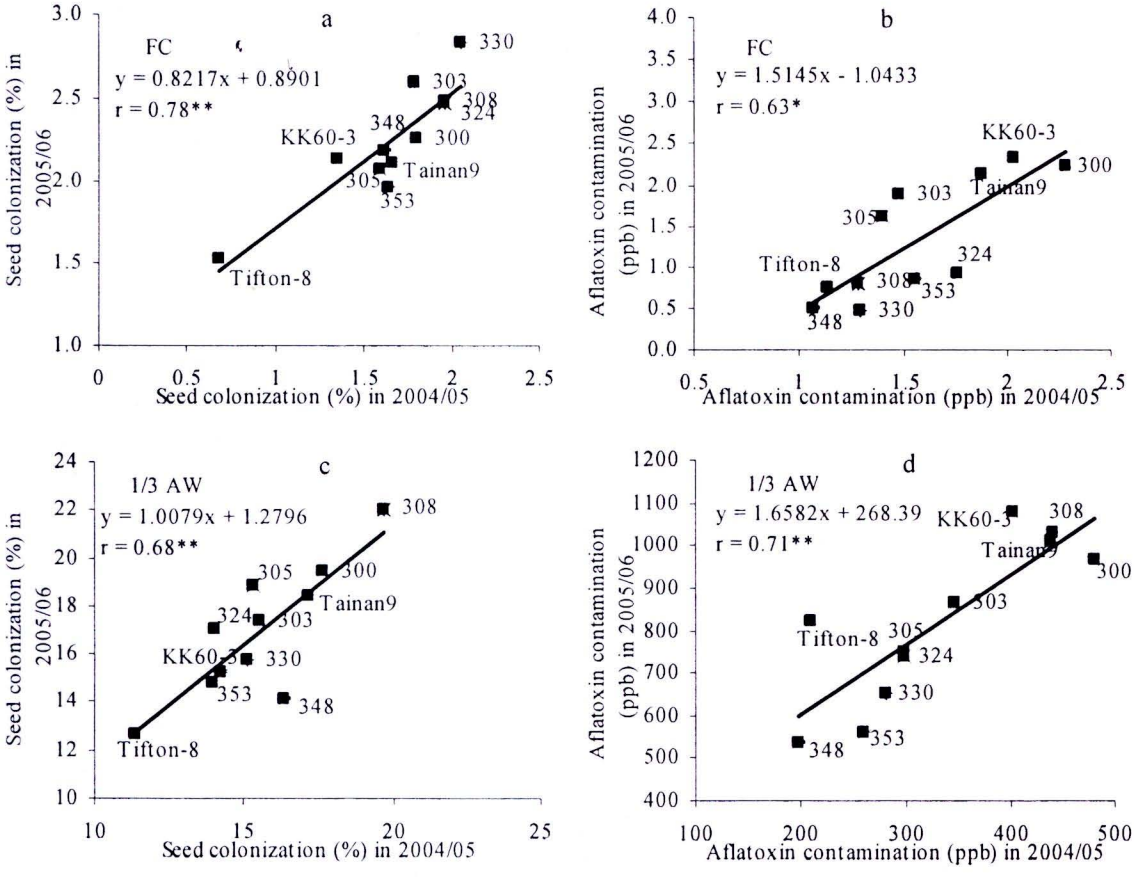

Figure 6 Genotypic performance of seed colonization by *A. flavus* under field capacity (FC) (a) and 1/3 available water (1/3 AW) (c) and genotypic performance of aflatoxin contamination under FC (b) and 1/3 AW (d) of 11 peanut genotypes in 2004/2005 and 2005/2006.

Correlations between physiological traits and *A. flavus* colonization and aflatoxin contamination

Close associations between physiological traits for drought resistance and *A. flavus* colonization and PAC were found across years (Table 3). Correlations between *A. flavus* colonization and PAC and surrogate traits for drought resistance were found under water stressed conditions, but were not found under well-watered conditions (data not shown). Correlations between DTI of biomass and *A. flavus* colonization and PAC were significant (ranged from r = -0.59* to r = -0.67*). These results revealed that peanut genotypes with an ability to maintain high biomass production under terminal drought also had relatively low aflatoxin production. Positive correlations between SLA, DSR, and canopy temperature and *A. flavus* colonization and PAC were significant (ranged from r = 0.58* to r = 0.77**). Negative and significant correlations between ChlD and RWC and *A. flavus* colonization and PAC (ranged from r = -0.57* to r = -0.75**) were also observed (Table 3).

Table 3 Correlation between *A. flavus* colonization (%) and aflatoxin contamination (ppb) and surrogate traits for drought tolerance of 11 peanut genotypes under terminal drought in 2004/05 and 2005/06 dry seasons.

Surrogate trait for drought tolerance	2004/05 (n=11)		2005/06 (n=11)	
	A. flavus	Aflatoxin	*A. flavus*	Aflatoxin
DTI of biomass	-0.67 *	-0.59 *	-0.52	-0.59 *
SLA	0.60 *	0.68 *	0.67 *	0.77 **
SCMR	-0.53	-0.35	-0.36	-0.53
ChlD	-0.67 *	-0.57 *	-0.60 *	-0.61 *
RWC	-0.71 *	-0.58 *	-0.75 **	-0.63 *
DSR	0.58 *	0.59 *	0.71 **	0.56
Canopy temperature	0.56	0.50	0.64 *	0.66 *

DTI, drought tolerance index; SLA, specific leaf area; ChlD, chlorophyll density; SCMR, SPAD chlorophyll meter reading; RWC, relative water content; DSR, drought stress ratings.

* and ** are significant at 0.05 and 0.01 level of probability, respectively.

The correlations between physiological traits for drought resistance and *A. flavus* colonization and PAC were consistent even thought the ranking of cultivars in *A. flavus* infection and aflatoxin contamination was quite different between the years. This indicated that breeding for low aflatoxin contamination in peanut might be achieved based on physiological-based selection for drought resistance.

Multiple regressions showed that the contribution of surrogate traits for drought tolerance to *A. flavus* colonization and PAC were not consistent across years (Table 4). RWC and ChlD contributed 50.9 % and 21.3 %, respectively, to *A. flavus* colonization in 2004/05 but these traits did not contribute to *A. flavus* colonization in 2005/06. In 2005/06, the contributions of surrogate traits for drought tolerance to *A. flavus* colonization were mainly from DSR (51.2 %). RWC contributed 34.0 % to PAC in 2004/05 and SLA contributed 58.9 % to PAC in 2005/06.

Table 4 Contribution of surrogate traits for drought tolerance to *A. flavus* colonization and aflatoxin contamination of 11 peanut genotypes under terminal drought in 2004/05 and 2005/06 dry seasons.

Surrogate trait for drought tolerance	Explained by regression (%) in 2004/05		Explained by regression (%) in 2005/06	
	A. flavus	Aflatoxin	*A. flavus*	Aflatoxin
Regression	82.6 **	58.1 *	74.3 *	72.8 *
SLA	7.0	0.6	20.5	58.9 *
ChlD	21.3 *	5.5	0.1	11.3
RWC	50.9 **	34.0 *	2.5	0.9
DSR	3.4	18.1	51.2 *	1.7

SLA, specific leaf area; ChlD, chlorophyll density; RWC, relative water content; DSR, drought stress ratings.

* and ** are significant at 0.05 and 0.01 level of probability, respectively.

Discussions

Heat and drought promoted the growth and persistency of *A. flavus* populations in soil. According to Blankenship et al. (1984) who observed that *A. flavus* grows very readily under high soil temperatures and low soil moisture contents, *Aspergillus* spp. are uniquely thermo-tolerant, and thrive during drought- and heat-stressed conditions. The temperature optimum for growth of *A. flavus* (25-42 ^{0}C) is higher than for many other species (Klich et al., 1992).

Drought-resistant genotypes were observed to have lower *Aspergillus* colonization and PAC. Tifton-8 which is a drought-resistant germplasm line had low seed colonization and PAC. These results are in agreement with the reports by Chenault et al. (2004) and Holbrook et al. (2000a) who found that Tifton-8 had some resistance to PAC. Tifton-8 had low visual stress rating (Rucker et al., 1995) and high phytoalexin (Sobolev et al., 2007) under water stress. However, Anderson et al. (1995) and Holbrook (2000b) did not observe a reduction in PAC in Tifton-8 compared with other genotypes. The drought-resistant peanut genotypes from

ICRISAT ICGV 98305, ICGV 98330, ICGV 98348, and ICGV 98353 also had relatively low PAC.

The relationships of increased drought tolerance and reduced aflatoxin production were confirmed by this study. It could be hypothesized that a genotype which has a greater level of drought tolerance would have lower PAC than a drought susceptible genotype. Correlation between DSR and aflatoxin production has been report in peanut by Holbrook et al. (2000a) who found significant positive correlations between PAC and visual stress ratings. They also found a negative correlation between PAC and yield under drought stressed conditions. In our study, DSR seems to be a fast and inexpensive tool, but the correlation to PAC was not consistent. Drought stress ratings might be used in combination with other physiological traits as indirect selection tools for lower aflatoxin contamination.

Genetic variation is needed for a trait to be successfully used as a surrogate for aflatoxin contamination under drought conditions. These findings indicated that SLA and RWC may be useful tools for selecting peanut genotypes with reduced *A. flavus* colonization and aflatoxin contamination based on their correlations with aflatoxin contamination and their variation. SLA can be an efficient tool for selecting peanut with drought tolerance in breeding programmes (Nageswara Rao and Wright, 1994; Wright et al., 1994). SLA and RWC are less variable and cheaper to measure than aflatoxin contamination. Moreover, these traits are stable across environments due to low G x E interactions (Wright et al., 1988; Nageswara Rao et al., 1995; 2001). Nautiyal et al. (2002) studied the relationship between SLA and RWC and observed that low SLA can help to maintain plant water status during drought and support metabolic activities to maintain favorable leaf temperature. The ability to maintain plant water status under drought can also help to maintain the capacity of peanut seeds to produce phytoalexin preventing aflatoxin production (Dorner et al., 1989; Wotton and Strange, 1985).

SLA is associated with variation in photosynthetic capacity (Wright and Nageswara Rao, 1994; Nageswara Rao et al., 1995). Therefore, peanut genotypes with higher leaf thickness or low SLA have more photosynthetic capacity. In addition, thicker leaves have thicker cuticles that can contain more chlorophyll pigment. Chlorophyll density was shown to have potential as an indirect selection tool for improving resistance to PAC in peanut breeding. This result was supported by

Arunyanark et al. (2008) who found that chlorophyll density can be an important factor contributing to drought resistance and transpiration efficiency which is a primary mechanism to avoid water loss in plants.

In conclusion, drought tolerance traits have the potential to serve as indirect selection tools for resistance to PAC. Drought-tolerant lines can still exceed the maximum permissible limit of PAC when tested under severe drought and heat stress. However, they do exhibit low contamination relative to drought-intolerant genotypes. SLA, RWC, ChlD, and DSR may be useful traits for indirect selection for lower aflatoxin contamination. These physiological traits can also be used as efficient tools for selection of peanut genotypes with terminal drought tolerance and low levels of PAC. DSR could be used as a rapid tool for screening for drought tolerance and reducing PAC in large peanut germplasms because it is inexpensive and easy to use. SLA and RWC seem to be the best surrogate traits for drought tolerance and low PAC based on low G x E interactions and good correlations to PAC. The genotypes ICGV 98305, ICGV 98330, ICGV 98348, and ICGV 98353 which are elite drought-resistant lines from ICRISAT, and Tifton-8 which is a drought resistant line from USDA were observed to have relatively low PAC in these studies.

Acknowledgments

The authors are grateful for the financial support of the Royal Golden Jubilee Ph.D. program (grant no. PHD/0208/2545) and the Senior Research Scholar Project of Professor Dr. Aran Patanothai under the support of Thailand Research Fund, and also support in part by the Peanut Improvement Project of Khon Kaen University. We thank the work of many people in field data collection and processing.