RESEARCH ARTICLE

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ASSOCIATION OF AFLATOXIN CONTAMINATION AND ROOT TRAITS OF PEANUT GENOTYPESUNDER TERMINAL DROUGHT

R. KOOLACHART[1], S. JOGLOY[1], N. VORASOOT[1], S. WONGKAEW[2], C.C. HOLBROOK[3], N. JONGRUNGKLANG[1], T. KESMALA[1] AND B. SURIHARN[1,*]

[1]Department of Agronomy, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand
[2]School of Crop Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand
[3]USDA-ARS, Coastal Plain Experiment Station, P.O. Box 748, Tifton 31793, Georgia, USA
*Corresponding author's email: sphala@kku.ac.th
Email addresses of coauthors: ratanaporn38@hotmail.com, sjogloy@gmail.com, nvorasoot1@gmail.com; nuntjo@kku.ac.th, thkesmala@gmail.com, swsopone@gmail.com, corley.holbrook@ars.usda.gov

SUMMARY

Aspergillus flavus colonization and aflatoxin contamination in peanut is one of the major problems of peanut production. The aflatoxin is carcinogenic and harmful to consumers. Drought resistant variety had low *A. flavus* infection and low aflatoxin contamination. The information on the association of root parameters and aflatoxin contamination in the end season drought is important for improvement of peanut cultivars for drought prone environments and reduction in pre-harvest aflatoxin contamination. This work aimed to investigate the association of root parameters and aflatoxin contamination of peanut in end of season drought. The split plot design was set up in the field. The two main plots were consisting of two water levels (field capacity (FC) and 1/3 available water (1/3 AW) from the R7 growth stage (R7) through harvest) and five subplots including five peanut varieties (ICGV 98308, ICGV 98324, ICGV 98348, Tainan 9 and Tifton 8). Data were recorded for *Aspergillus flavus* colonization, aflatoxin contamination, percent root length density, root surface, root diameter and root volume in the deeper soil layer (30-90 cm) at harvest. ICGV 98348 and ICGV 98324 were the best varieties for low aflatoxin contamination, low *A. flavus* colonization, and high roots traits (percent root length density, root surface, root diameter and root volume). Our studies demonstrated that roots traits may be important traits related to aflatoxin contamination and confirmed that colonization is related to aflatoxin contamination. Root traits may be useful alternative selection criteria for reduced aflatoxin contamination under terminal drought.

Key words: *A. flavus* colonization, drought tolerance, end season drought, low aflatoxin contamination, water regime

Key findings: Peanut varieties with high root traits in the deeper soil layer and low *A. flavus* colonization could maintain low aflatoxin contamination. Roots traits may be useful alternative selection criteria for reduced aflatoxin contamination under terminal drought.

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INTRODUCTION

Aspergillus flavus produce aflatoxin and contaminate in peanut and its products (Amaike and Keller, 2011). Aflatoxin contamination is also an issue of trade barriers in several countries. Consumption of contaminated food is a serious cause of health in human and livestock (Roze *et al*., 2013). In peanut, end of season drought causes the most severe yield loss and aflatoxin contamination (Girdthai *et al.*, 2010a; Girdthai *et al.*, 2010b; Holbrook *et al.*, 2000). Increased duration of end of season drought and temperature are major factors determining the level of aflatoxin contamination (Waliyar *et al.*, 2003b). Because at the pod filling stage, drought cause plant stress and produce lower phytoalexin (Dorner *et al.*, 1989) then *A. flavus* can colonize in peanut pods prior to harvest and the contamination of aflatoxin is more severe under end of season drought (Waliyar *et al.*, 1994).

Root is important for water acquiring in drought condition root distribution may be important as selection tools for drought resistance (Matsui and Singh, 2003; Taiz and Zeiger, 2006). Root is the subterranean plant part that functions as a plant anchor and takes up water and nutrients. Ability of root growth is important for peanut to survive under drought conditions (Rucker *et al.*, 1995). Adaptation of plant for root traits as affected by drought is an important mechanism of drought resistance. Under drought conditions, the plant develops deeper roots to mine more water and to avoid desiccation as a mechanism of drought avoidance. In our previous investigation, peanut varieties subjected to drought during late growth stages had different levels of resistance to aflatoxin contamination (Girdthai *et al.*, 2010a), and, therefore, peanut genotypes with contrasting responses to end of season drought for root traits were tested in this study. The assumption underlying this research project is whether adaptation of peanut genotypes for root characters under terminal drought contributes to alleviate stress in plant and to lower aflatoxin contamination or not. If the assumption is confirmed in this experiment, the responses to end of season drought for root traits might be related to low aflatoxin contamination.

The knowledge on the association of aflatoxin contamination and root parameters in end of season drought condition has not been well

established in peanut. The knowledge on the association of aflatoxin contamination and root parameters can be used in peanut breeding for drought prone environments and reduced pre-harvest aflatoxin contamination. Thus, selection for root traits might increase selection efficiency and improve resistance to pre-harvest aflatoxin contamination. Arunyanark *et al.* (2009) found significant association in aflatoxin contamination and root traits in long term drought of peanut. However, aflatoxin contamination during terminal drought in peanut is a severe problem then information on the association of root parameters and aflatoxin contamination in terminal drought should be investigated. The goal of present study was to determine the association in root parameters of peanut genotypes with aflatoxin contamination of peanut varieties having different levels of terminal drought resistance.

MATERIALS AND METHODS

Genetic material

The peanut varieties were selected due to their contrasting levels of aflatoxin contamination under terminal drought in our previous study (Girdthai *et al.*, 2010a). ICGV 98324 and ICGV 98348 had low aflatoxin contamination while ICGV 98308 and Tainan 9 had high aflatoxin contamination. The ICGV accessions were kindly donated from ICRISAT (Nageswara *et al.*, 1994; Nigam *et al.*, 2003; Nigam *et al.*, 2005), and they were classified as drought resistant varieties (Girdthai *et al.*, 2010a). The descriptions for tested varieties in present work were reported in our parallel study (Koolachart *et al.*, 2013).

Experimental design

A split-plot design with four replications for two consecutive years (2010 and 2011) was set up in field conditions at Khon Kaen University, Thailand. Two water levels [field capacity (FC) and 1/3 available water (1/3 AW) from the R7 growth stage through harvest] were applied in main-plots and 5 peanut varieties (ICGV 98308, ICGV 98324, ICGV 98348, Tainan 9 and Tifton 8) were arranged as sub-plots. Peanut was grown in the plots with 5 × 5 m and a spacing of 50 ×20 cm. The crop relied solely on irrigated water.

Crop management

The details for crop management were available in our parallel study (Koolachart *et al.*, 2013), and brief descriptions of crop management were reported herein. The soils at the experimental sites were prepared conventionally to provide suitable soil physical properties and the fertilizers for phosphorus and potassium were incorporated during soil preparation. Fungicide with active compound Captan was used to treated seeds to control soil born disease and provide higher germination.

The seeds were grown and thinned to obtain one plant per hill at 14 days after planting (DAP). Inoculation of *Rhizobium* was carried out soon after planting and pre-emergent herbicide was applied soon after planting.$CaSO_4$was supplied at 45 DAP to provide sufficient calcium to the developing pods. Insect pests and diseases were properly control to provide better control of the crop.

Water management

The detailed descriptions of water management are available in the parallel study of our project (Koolachart *et al.*, 2013). The irrigated water was supplied through a sub-surface drip irrigation system. A pressure valve and a water meter were installed for each main plot, and a sub-valve was also installed for each sub-plot. The FC was maintained uniformly at FC level from the soil surface to 60 cm below the soil surface from planting to harvest, whereas 1/3 AW level was commenced at R7 growth stage until harvest. The replenishment of irrigated water was carried out by adding water to the plots according to crop water requirement (Doorenbos and Pruitt, 1992) and surface evaporation (Singh and Russel, 1981).

Meteorological conditions, soil properties and soil moisture content

Meteorological data were recorded daily for relative humidity, pan evaporation, rainfall, maximum and minimum air temperature and solar radiation throughout the experiment duration by the nearest weather station.

The soil from four positions in the field at the depths of 0-5, 5-15 and 15-30 cm from the soil surface were bulked into a single pile for each layer. The soil samples were then analyzed for physical and chemical properties prior to planting. The soil is Yasothon soil series with loamy sand texture in Year 1 and sand texture in Year 2 (Table 1).

Gravimetric method was used for soil moisture content at planting, last day of irrigation, R7 and at harvest at 0-5, 10-15, 25-30, 40-45 and 55-60 cm below the soil surface. The soils were sampled at two positions in the plots.

***A. flavus* colonization and aflatoxin contamination**

The *A. flavus* was donated by Suranaree University of Technology, Nakhon Ratchasima, Thailand. The strain was able to produce aflatoxin in peanut and it was maintained on potato dextrose agar (PDA). In preparation of the inoculums, the fungus was cultured in PDA medium for ten days. It was transferred onto the new medium consisting of ground peanut and corn and incubated at 25-30 °C for 14 days before being used as inoculum. The inoculum was spread onto the experimental plots at the rate of 375 kg ha^{-1} at 30 DAP.

Data were recorded for *A. flavus* population, *A. flavus* colonization and aflatoxin contamination according to the methods described previously (Girdthai *et al.*, 2010b; Arunyanark *et al.*, 2009). Soil samples at six positions in the main plots were collected at harvest and bulked into a single pile. Then, one kg of soil samples was used for measuring *A. flavus* population.

The soil samples were stored in the sterilized plastic bags and transported to the laboratory for plate count in an *A. flavus* selective medium. The sub-sample of 100 g was mixed with 125 ml of sterilized water and stirred in a stirrer for 1 min. Water was added to the sample to obtain the final dilution factor of 1:10 dilution factor and 100 µl of the sample was spread on the surface of the selective Rose Bangal agar. The plates were then incubated at 25 °C, for 7 days and green colonies of *A.*

Table 1. Chemical and physical properties of the soil at the depth 0-30 cm, soil moisture content (%) at the depth 0-60 cm at sowing, at the last day of irrigation, R7 stage and harvest stage and population of *Aspergillus flavus* in soil at harvest under field capacity (FC) and 1/3 available water (1/3 AW) in the dry seasons 2010/2011 (Year 1) and 2011/2012 (Year 2).

Chemical and physical properties of the soil

Years	pH (1:1 H_20)	EC (1:5 H_20) (dSm^{-1})	CEC ($cmolkg^{-1}$)	OM (%)	Total N (%)	Available P ($mgkg^{-1}$)	Exchangeable		Particle size, mμm (USDA system)			Texture class
							K ($mgkg^{-1}$)	Ca ($mgkg^{-1}$)	Sand: 2.0-0.05 (%)	Silt: 0.05-0.002 (%)	Clay: <0.002 (%)	
Year 1	6.08	0.03	5.22	0.44	0.02	23.95	33.09	418.33	85.08	7.30	7.62	Loamy sand
Year 2	6.18	0.05	5.93	0.41	0.01	40.74	38.34	446.67	89.99	8.32	1.70	Sand

Soil moisture content (%) and population of *A. flavus* in soil(colonies/gram of soil)

Years	Treatments	Soil moisture content (%)				Population of *A. flavus*in soil (CFU/1 g of soil)
		Sowing	At the last day of irrigation	R7 stage	Harvest	Harvest
Year 1	FC	10.33	7.67	8.24	9.51	7.50b
	1/3 AW	10.26	7.47	5.91	6.43	14.69a
Year 2	FC	10.81	10.63	10.83	10.76	5.31b
	1/3 AW	10.42	10.42	6.12	6.44	10.31a

Year 1: FC = 10.14 %, PWP = 4.47, 1/3 AW = 6.33; Year 2: FC = 10.18 %, PWP = 4.50, 1/3 AW = 6.37 using pressure plate method. Means within a column followed by the same letter are not significantly different (at $P \leq 0.05$) by DMRT for each year.

flavus were counted. The data were reported as colony forming units (CFU) per 1 g of soil.

A. flavus colonization in each plot was determined from 100 dry seeds with approximately 8% moisture content. The seeds were surface-sterilized in a 10% aqueous solution of Clorox (0.525% NaOCl) for 2 min, rinsed in sterilized water and incubated in moist paper for five days at room temperature (25-30°C). Then, green conidial heads of *A. flavus* were counted on the seeds and the data were reported as percent of colonized seeds.

A sample of 100 seeds in each plot was taken for analysis of aflatoxin contamination using a competitive Enzyme Linked Immunosorbent Assay (ELISA) method. The details of aflatoxin analysis (B_1) were reported previously (Arunyanark *et al.*, 2009).

Root trait observation

Root samples were recovered from the soil at harvest using an auger [26, 27] at the depths of 0-15 cm, 15-30 cm, 30-45 cm, 45-60 cm, 60-75 cm and 75-90 cm below the soil surface. The roots were sampled at the center of plant and at between rows. The samples were washed to remove soil. Root samples were then scanned and analyzed with the Winrhizo program (Winrhizo Pro (s) V. 2004a, Regent Instruments, Inc) to determine total root length, root surface area, root diameter and root volume per sample.

Root length density (RLD) was calculated as the ratio of root length and soil volume. Root traits at 0-15 and 15-30 cm were combined into an upper soil layer, and root traits at 30 to 90 cm were combined as a lower soil layer. For each peanut variety the relative contribution of RLD in the lower layers was calculated and defined as percent of root length density (% RLD) in the 30-90 cm soil layer. Drought tolerance indexes (DTI) of each root traits were calculate as the ratio of root traits under water stress and root traits under non-stress conditions.

Statistical analysis

Data were statistical analysis was conducted using MSTAT-C software (Bricker, 1989). The data were first analyzed for individual year, and the error variances were tested for variance homogeneity by Bartlett's test (Hoshmand, 2006). The data of two years were combined for the traits with variance homogeneity, and means were separated by Duncan's multiple range test (DMRT). The association between root traits in the lower soil layer and aflatoxin colonization and contamination were investigated by plotting the traits in Microsoft Excel.

RESULTS

Meteorological conditions

The relative humidity ranged from 69 to 95%and 71 to 91%, the total amounts of rainfall were 81.1 and 41.4 mm, and means of daily pan evaporation were 4.47 and 5.14 mm in Year 1 and Year 2, respectively (Figure 1a and 1c). Rainout shelters were used when it rained. Maximum and minimum temperatures were different between years. Temperatures in Year 1 and Year 2 were 14.0-34.4 °C and 12.0-37.0 °C, respectively and the yearly means of solar radiation were 13.23 and 19.18 MJ m^{-2} day^{-1} for

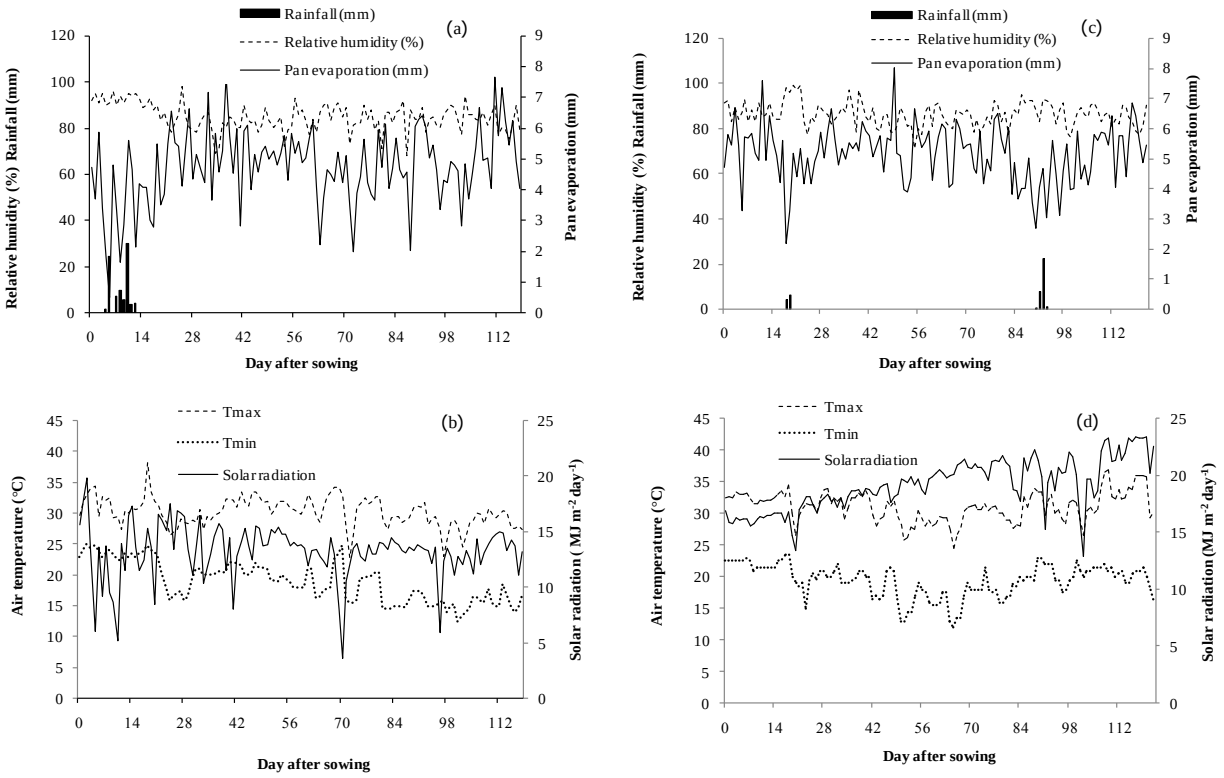

Figure 1. Rainfall, relative humidity (RH), evaporation (E0), maximum (Tmax) and minimum (Tmin) temperatures and solar radiation during October-February 2010/2011 (Year 1; a, b) and 2011/2012 (Year 2; c, d) at the meteorological station, KhonKaen University, Thailand.

Year 1 and Year 2, respectively (Figure 1b and 1d).

Soil moisture content and relative water content (RWC)

Soil moisture contents of stressed and non-stressed treatments were maintained at field capacity at sowing for both years and at the last day of irrigation for Year 2 (Table 1). Crop water requirements in Year 1 were calculated at initial phase of drought treatment based solely on ET crop. The crop in Year 1 was more stressed although drought was not commenced, indicating that the replenishment of irrigated water to the crop is not sufficient. In Year 2, ET crop plus Es was added in the calculation of water requirement.

Stressed treatment and non-stressed treatments in both years were different for relative water content at the R7 and harvest. Higher relative water content was observed in non-stressed crop than in stressed crop in both years (data not shown).

Soil temperature

Peanut genotypes were not significantly different for soil temperature under non-water stressed and water stressed treatments at the last day of irrigation, but the water-

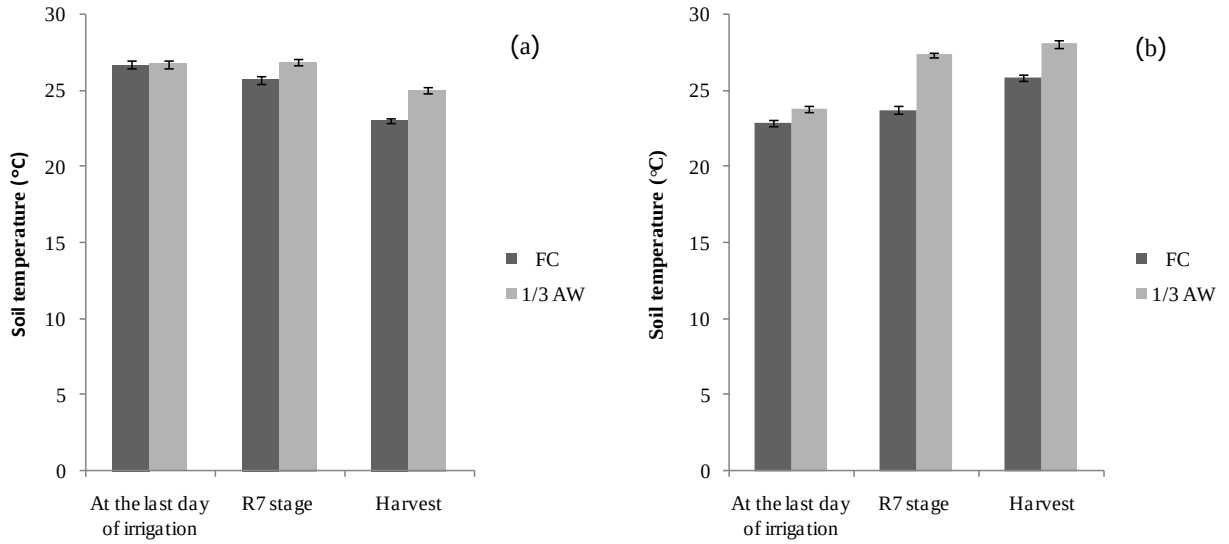

Figure 2. Soil temperature at the last day of irrigation, R7 stage and harvest for peanut grown under field capacity (FC) and 1/3 available water (1/3 AW) in the dry seasons 2010/2011 (Year 1; a) and 2011/2012 (Year 2; b).

stressed treatment had higher soil temperature than did non-water stressed treatment at the R7 and at harvest in both years (Figure 2). As crop water requirement was adjusted after drought imposition to the crop, soil temperature in Year 1 reduced slightly from the last day of irrigation until harvest under both conditions, whereas the soil temperature in Year 2 increased slightly from the last day of irrigation until harvest. This may be due to the difference of soil moisture content between years. In Year 1 after withholding water for 10 days, additional water was added for controlling soil moisture content.

Soil populations of *A. flavus*

Water levels were significantly different for soil population of *A. flavus* at final harvest in both years (Table 1). The soil exposed to drought had higher *A. flavus* populations (14.69 and 10.31 colonies/g soil in Year 1 and 2, respectively) than did well-irrigated soil (7.50 and 5.31 colonies/g soil in Year 1 and 2, respectively). In lower soil moisture, it had lower water status in crop plants (data not shown), but higher *A. flavus* population (Table 1).

Combined analysis of variance

Water levels were significantly different of all parameters and the differences among peanut varieties were also significant (Table 2). Years were also significantly different for most parameters except for root diameter.

The interactions between year and water regime and water regime and varieties were significant for most parameters except for aflatoxin contamination and root diameter, and the interactions between water regime and year were significant for most parameters except for aflatoxin contamination and root diameter,

Table 2. Mean squares from combined analysis of *Aspergillus flavus* colonization on seed, aflatoxin contamination in seed at harvest, % RLD, root surface area, root diameter and root volume in the deeper soil layer (30-90 cm) of five peanut genotypes grown under field capacity (FC) and 1/3 available water (1/3 AW) in the dry seasons 2010/2011 (Year 1) and 2011/2012(Year 2).

Source	d.f.	*A. flavus* colonization	Aflatoxin contamination	% RLD	Root surface area	Root diameter	Root volume
Year (Y)	1	673.96**(27.46)	2319.10**(55.63)	917.19**(9.85)	27169.20**(37.68)	0.02^{ns}(1.15)	2.89**(32.71)
Rep within Year	6	6.03(1.47)	9.77(1.41)	16.19(1.04)	35.2(0.29)	0.01(2.96)	0.00(0.13)
Water regimes (W)	1	660.10**(26.90)	256.79**(6.16)	1415.98**(15.20)	7784.10**(10.80)	0.13** (6.61)	1.19**(13.48)
Y × W	1	92.02**(3.75)	0.36^{ns}(0.01)	1242.49**(13.34)	6235.30**(8.65)	0.02^{ns}(0.96)	0.87**(9.78)
Error (b)	6	3.76(0.92)	13.32(1.92)	8.4 0(0.54)	19.80(0.16)	0.01(1.71)	0.01(0.65)
Genotypes (G)	4	119.59**(19.49)	121.09**(11.62)	417.50**(17.93)	1220.60**(6.77)	0.20**(40.51)	0.26**(11.64)
Y × G	4	33.88**(5.52)	74.63**(7.16)	154.39**(6.63)	430.50**(2.39)	0.02^{ns}(4.33)	0.02^{ns}(1.07)
W × G	4	37.13** (6.05)	23.81^{ns}(2.28)	380.85**(16.36)	2993.40**(16.61)	0.004*(8.24)	0.23**(10.37)
Y × W × G	4	3.75^{ns}(0.61)	3.64^{ns}(0.35)	255.28**(10.96)	2989.60**(16.58)	0.01^{ns}(2.13)	0.33**(14.79)
Error (c)	48	4.00(7.82)	11.69(13.47)	15.79(8.14)	36.30(0.07)	0.01(31.40)	0.01(5.36)
CV (%) (a)		38.45	37.74	13.91	8.83	6.83	6.12
CV (%) (b)		36.55	43.92	9.48	6.62	5.19	13.58
CV (%) (c)		37.72	41.16	12.99	8.96	7.86	13.77

d.f.: degree of freedom, *C.V.*: coefficient of variation, *% RLD*: percentage of root length density ((RLD 30to 90 cm/ RLD 30 to 90 cm +RLD 0 to 30 cm) × 100). ns, *, ** non-significant and significant at $P \leq 0.05$ and $P \leq 0.01$ probability levels, respectively. Values in parenthesis are percentages of sum squares.

Table 3. *Aspergillus flavus* colonization on seed and aflatoxin contamination in seed at harvest of five peanut genotypes grown under field capacity (FC) and 1/3 available water (1/3 AW) in the dry years 2010/2011 (Year 1) and 2011/2012 (Year 2).

Genotypes	*A. flavus* colonization (%)				Aflatoxin contamination (ppb)			
	Year 1		Year 2		Year 1		Year 2	
	FC	1/3 AW	FC	1/3 AW	FC	1/3 AW	FC	1/3 AW
ICGV 98308	0.00c	7.00a	1.00b	8.75a	12.78ab	18.93ab	7.85a	16.85a
ICGV 98324	1.00ab	1.25c	3.50a	3.50b	10.80bc	14.25bc	4.10bc	9.75b
ICGV 98348	0.00c	0.50c	1.00b	2.00b	6.80c	7.63c	0.00d	4.10c
Tainan 9	1.50a	4.25b	0.00c	5.25ab	17.85a	22.50a	6.30ab	16.05a
Tifton 8	0.50bc	8.00a	0.50bc	3.75b	10.95bc	14.45bc	3.05c	14.00ab
Mean	0.60	4.20	1.20	4.65	11.84	15.55	4.26	12.20

Means within a column followed by the same letter are not significantly different at $P \leq 0.05$probability levels by DMRT.

whereas the interactions between year and peanut genotype were significant for most parameters except for root diameter and root volume. Significant G × E interactions indicated differential responses among peanut varieties to environmental changes.

The interactions among years, water levels, and peanut varieties were not significant for most parameters except for % RLD, root surface area and root volume in the 30-90 cm soil layer. Water levels contributed to large percentages of variations in *A. flavus* colonization (26.90%), % RLD in the 30-90 cm soil layer (15.20%), root volume (13.48%) and root surface area (10.80%), whereas varieties contributed to large percentages of variations in root diameter (40.51%), *A. flavus* colonization (19.49%), % RLD in the 30-90 cm soil layer (17.93%), aflatoxin contamination (11.62%) and root volume (11.64%). The contribution of G x W interactions was smaller than that of water regimes except for % RLD in the 30-90 cm soil layer (16.36%) and root surface area (16.61%).

Effects of terminal drought on *A. flavus* colonization and Aflatoxin contamination in the 30-90 cm soil layer

Significant differences of varieties were met for *A. flavus* colonization and aflatoxin contamination for non-stressed and stressed treatments at harvest for both years (Table 3). Tifton 8 and ICGV 98308 had the highest *A. flavus* colonization under stressed conditions (8.00% and 7.00%, respectively), whereas ICGV 98348 and ICGV 98324had the lowest *A. flavus* colonization under stressed conditions (0.50% and 1.25%, respectively) in Year 1. ICGV 98308 and Tainan 9 had the highest *A. flavus* colonization under stressed conditions (8.75% and 5.25%, respectively), whereas ICGV 98348 and ICGV 98324 had the lowest *A. flavus* colonization under stressed conditions (2.00% and 3.50%, respectively) in Year 2. Under terminal drought, *A. flavus* colonization ranged from 0.50% to 8.00% in Year 1 and from 2.00% to 8.75% in Year 2.

Tainan 9 and ICGV 98308 had the highest aflatoxin contamination under stressed conditions (22.50 ppb and 18.93 ppb, respectively), whereas ICGV 98348 and ICGV 98324had the lowest aflatoxin contamination under stressed conditions (7.63 ppb and 14.25 ppb, respectively) in Year 1(Table 3). ICGV 98308 and Tainan 9 had the highest aflatoxin contamination under stressed conditions (16.85 ppb and 16.05 ppb, respectively) in Year 2, whereas ICGV 98348 and ICGV 98324 had the lowest aflatoxin contamination (4.10 ppb and 9.75 ppb, respectively).

Effects of terminal drought on % RLD and root surface area in the 30-90 cm soil layer

Significant differences of varieties in both years were observed for % RLD and root surface area in the deeper soil layer (30-90 cm) in stressed and non-stressed treatments at harvest (Table 4). Most DTI were higher than one. Most varieties were rather similar for % RLD and root surface area in the 30-90 cm soil layer in non-stressed treatments except for Tifton 8, which had rather low % RLD and root surface area in the 30-90 cm soil layer in stressed treatment. In contrast to Tifton 8, other varieties showed high % RLD and root surface area in the

Table 4. Percent RLD and root surface area in the deeper soil layer (30-90 cm) at harvest of five peanut genotypes grown under field capacity (FC) and 1/3 available water (1/3 AW) in the dry seasons 2010/2011 (Year 1) and 2011/2012 (Year 2).

Genotypes	% RLD						Root surface area (cm^2)					
	Year 1			Year 2			Year 1			Year 2		
	FC	1/3 AW	DTI	FC	1/3 AW	DTI	FC	1/3 AW	DTI	FC	1/3 AW	DTI
ICGV 98308	37.22ab	36.00a	0.98b	19.44b	36.09ab	1.86c	89.99b	87.90c	0.98b	41.23a	68.13ab	1.66c
ICGV 98324	31.60b	36.60a	1.20b	29.39a	40.15a	1.37c	74.74c	89.90bc	1.21bc	46.09a	77.65ab	1.69c
ICGV 98348	35.14b	39.10a	1.12b	28.73a	41.79a	1.45c	80.18c	107.39a	1.35b	31.81b	78.62a	2.59bc
Tainan 9	20.92c	41.51a	2.01a	7.76c	32.44b	4.18a	47.96d	104.79ab	2.20a	9.61d	49.87c	5.19a
Tifton 8	44.60a	18.45b	0.42c	9.89c	26.21c	2.65b	130.20a	43.45d	0.34d	21.76c	63.17bc	2.95b
Mean	33.89	34.23	1.01	19.04	35.34	1.85	84.61	86.69	1.02	30.10	67.49	2.24
CV (%)	15.78	11.65	19.91	8.90	11.35	15.51	4.90	8.99	10.76	14.04	10.42	15.45

DTI: Drought tolerance index (stress (1/3 AW)/ non-stress (FC); more than 1 = increased, less than 1 = decreased). Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ probability levels by DMRT.

Table 5. Root diameter and root volume in the deeper soil layer (30-90 cm) at harvest of five peanut genotypes grown under field capacity (FC) and 1/3 available water (1/3 AW) in the dry seasons 2010/2011 (Year 1) and 2011/2012 (Year 2).

Genotypes	Root diameter (mm) [1]						Root volume (cm^3)[1]					
	Year 1			Year 2			Year 1			Year 2		
	FC	1/3 AW[a]	DTI[a]	FC	1/3 AW	DTI	FC	1/3 AW	DTI	FC	1/3 AW	DTI
ICGV 98308	1.39ab	1.28	0.92	1.41ab	1.33b	0.95b	0.86b	0.84ab	0.99b	0.40ab	0.69ab	1.75c
ICGV 98324	1.45a	1.56	1.08	1.53a	1.66a	1.09ab	0.91b	1.09a	1.22b	0.46a	0.92a	2.01bc
ICGV 98348	1.54a	1.52	1.00	1.43a	1.60a	1.13ab	0.96b	1.03a	1.08b	0.34bc	0.91a	2.84bc
Tainan 9	1.27b	1.47	1.17	1.24b	1.37b	1.11ab	0.42c	1.11a	2.65a	0.08d	0.47b	6.29a
Tifton 8	1.48a	1.55	1.05	1.53a	1.74a	1.14a	1.33a	0.59b	0.44c	0.26c	0.81a	3.12b
Mean	1.43	1.48	1.03	1.43	1.54	1.08	0.91	0.93	1.02	0.31	0.76	2.45
CV (%)	7.82	9.17	11.12	7.61	6.72	7.57	6.79	14.57	17.43	14.48	16.42	17.65

DTI: Drought tolerance index (stress (1/3 AW)/ non-stress (FC); more than 1 = increased, less than 1 = decreased). [a] non-significant at $P \leq 0.05$ probability levels. Means within a column followed by the same letter are not significantly different at $P \leq 0.05$probability levels by DMRT.

30-90 cm soil layer in stressed treatment in Year 1. This study, drought increased % RLD and root surface area in the 30-90 cm soil layer for all varieties in Year 2 when compared with non-stressed treatment and for most varieties in Year 1 except for Tifton 8 and ICGV 98308.

Tainan 9 had the highest DTI for % RLD and root surface area in the 30-90 cm soil layer in both years (Table 4). It seems likely that Tainan 9 has better adaptation for root length density and root surface area in response to drought. ICGV 98324 and ICGV 98348 had high % RLD, root surface area in the 30-90 cm soil layer in stressed treatment in both years.

Effects of terminal drought on root diameter and root volume in the 30-90 cm soil layer

Significant differences of varieties in both years were observed for root diameter and root volume in the deeper soil layer (30-90 cm) under stressed and non-stressed treatments at harvest except for root diameter in stressed treatment in Year 1 (Table 5). Drought tended to increase root diameter and root volume in both years. Most DTI were higher than one and varieties were significantly different for this trait except for root diameter in Year 1. Tainan 9 had the highest DTI of root volume in both Year 1 (2.65) and Year 2 (6.29). Most varieties were rather similar for root diameter and root volume in non-stressed treatment except for root volume of Tifton 8 in Year 1 and Tainan 9 in Year 2, which had rather low root volume.

Relationships between root traits in the deeper soil layer (30–90 cm) with *A. flavus* colonization and aflatoxin contamination under stressed conditions

Root traits such as % RLD, root surface area, root diameter and root volume in the deeper soil layer (30-90 cm) were plotted against *A. flavus* colonization and aflatoxin contamination using means of each variety to examine how root traits influence *A. flavus* colonization and aflatoxin contamination under terminal drought conditions. Four quadrants such as low-low, low-high, high-low and high-high were used to explain the relationships between traits (Figure 3). The data are reported separately for each season to observe the consistency of the relationships. Theoretically, the peanut varieties with high root traits are expected to have low *A. flavus* colonization and aflatoxin contamination. This is because increased water uptake by roots should reduce the effects of drought stress, and may, therefore, reduce *A. flavus* colonization and aflatoxin contamination.

ICGV 98348 and ICGV 98324 were in quadrant with low *A. flavus* colonization and aflatoxin contamination in stressed conditions in both years, and they also had high % RLD in the 30-90 cm soil layer in both years (low-high quadrant) (Figure 3). Tifton 8 showed low aflatoxin contamination in Year 1 only, and it also had low % RLD in the 30-90 cm soil layer (low-low quadrant). Other peanut varieties did not show low *A. flavus* colonization and aflatoxin contamination in both years although

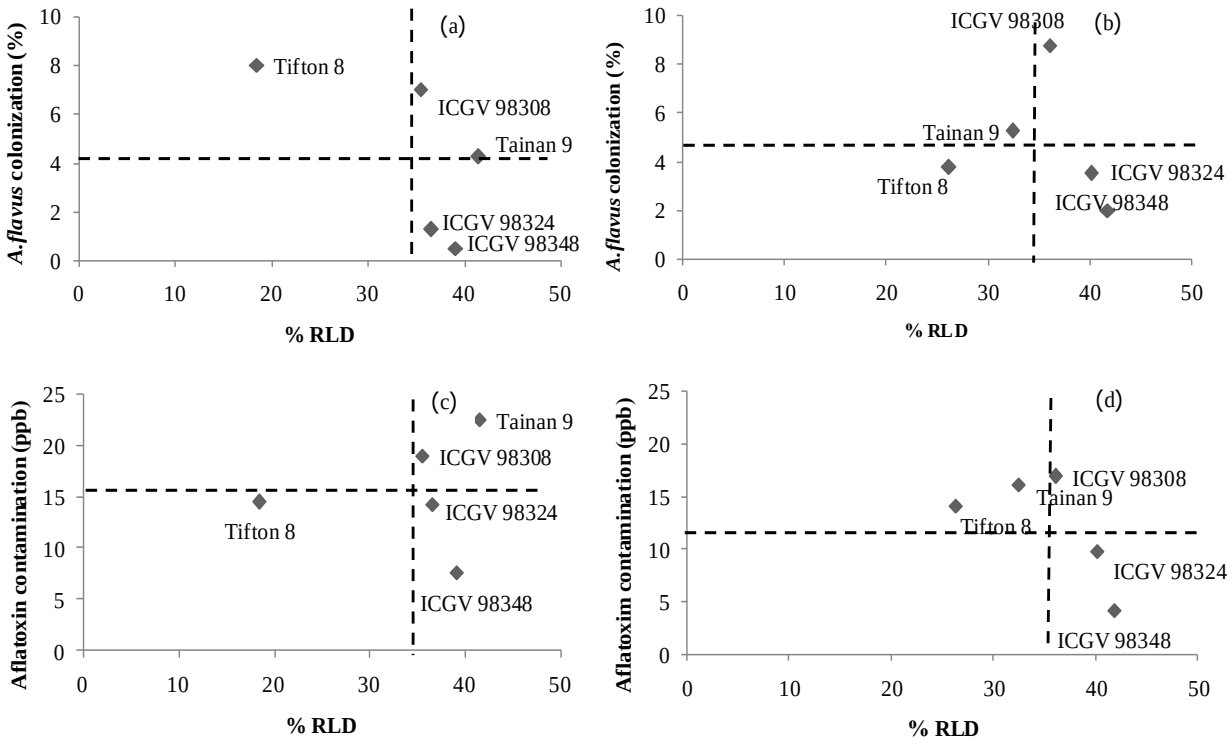

Figure 3. Relationships between % RLD in the deeper soil layer (30-90 cm) and *Aspergillus flavus* colonization (a), and aflatoxin contamination (c) in 2010/2011 (year 1) and relationships between % RLD in the deeper soil layer (30-90 cm) and *Aspergillus flavus* colonization (b) and aflatoxin contamination (d) in 2011/2012 (year 2) at harvest for five peanut genotypes grown under 1/3 available water (1/3 AW).

they had high % RLD in the 30-90 cm soil layer. The results showed that ICGV 98348 and ICGV 98324 had potential for resistance *A. flavus* colonization and low aflatoxin contamination. This may be due to high % RLD in the 30-90 cm soil layer.

The associations of root surface area, root diameter and root volume in the 30-90 cm soil layer with *A. flavus* colonization and aflatoxin contamination in stressed treatment were similar to that for % RLD in 30-90 cm soil layer (data not shown).

DISCUSSION

In this study, drought stress resulted in higher soil temperature at the R7 and at harvest in seasons, and higher *A. flavus* population, colonization and aflatoxin contamination. In general, drought affects *A. flavus* colonization and aflatoxin contamination in peanut depending on growth phases of peanut, the duration of drought, the severity of drought, and the temperature (Waliyar *et al.*, 2003b). Guo et al. (2003) stated that drought and high temperatures are conducive

to *A. flavus* colonization and aflatoxin contamination.

The present study showed that soil exposed to drought had higher *A. flavus* populations than did well-irrigated soil. In previous studies, terminal drought resulted in higher *A. flavus* populations in soils than well-irrigated conditions (Girdthai *et al*., 2010a), and long-term drought promoted the growth and persistence of the *A. flavus* population in soils (Arunyanark *et al*., 2009). It is then concluded that drought at most conditions increases *A. flavus* population in soil.

Our results showed that drought increased *A. flavus* colonization and aflatoxin contamination in both years, and there were significant variety by environment interactions for *A. flavus* colonization and aflatoxin contamination. Aflatoxin contamination in peanut is greatly influenced by environmental conditions (Girdthai *et al*., 2010b). Environmental variation and environment by variety interactions frequently results in inconsistent resistance of peanut genotypes across environments (Girdthai *et al*., 2010b; Anderson *et al*., 1995; Anderson *et al*., 1996; Holbrook *et al*., 1994). The results from the present study supported previous findings and indicated that direct selection for low *A. flavus* colonization and aflatoxin contamination in peanut is difficult. The interactions between year and peanut variety for % RLD and root surface area in the deeper soil layer were also significant ($P \leq 0.01$) and contributed to large portions of total variations. In previous investigation, Y × G interaction was significant for % RLD 40-100 cm ($P \leq 0.05$) under long term drought (Songsri *et al.*, 2008) whereas, Puangbut *et al*. (2009) reported that Y × G interaction was not significant for root dry weight under early season drought. High interactions may preclude their use as surrogate traits for low aflatoxin contamination under drought. However, high interaction also indicates the importance of multiple year evaluation of peanut varieties for root traits, and peanut varieties with consistent performance could be then identified.

In previous investigations, drought increased *A. flavus* colonization and aflatoxin contamination of peanut (Girdthai *et al*., 2010a; Arunyanark *et al*., 2009). Peanut varieties subjected to drought had high *A. flavus* colonization and aflatoxin contamination due to lower production of phytoalexin that inhibits the growth of *A. flavus* (Cole *et al*., 1985).

In different drought conditions in an earlier study, ICGV 98324 and ICGV 98348 had low aflatoxin contamination, whereas ICGV 98308 and Tainan 9 had high aflatoxin contamination (Girdthai *et al*., 2010a). In this study, ICGV 98324 and ICGV98348 had rather low *A. flavus* colonization andaflatoxin contamination in both years, although aflatoxin contamination in Tifton 8 was not different from ICGV 98308 and Tainan 9 in Year 2. However, low aflatoxin contamination in Tifton 8 has been reported by Holbrook *et al*. (2000) and Chenault *et al.* (2004).

Aflatoxin contamination in drought condition ranged from 7.63-22.50 ppb in Year 1 and from 4.10-16.85 ppb in Year 2. Higher aflatoxin contamination in Year 1 may be due to higher soil temperature. Increased aflatoxin contamination was found in drought-treated peanuts with increased soil temperatures (Cole *et*

al., 1985), and higher soil temperature favors *A. flavus* growth and aflatoxin production (Dorner *et al.*, 1989).

Our results showed that ICGV 98324 and ICGV 98348 had high % RLD, root surface area, root diameter and root volume in the deeper soil layer (30-90 cm) in stressed treatment in both years. The results indicated that the varieties had high ability to maintain root traits in the deeper soil layer under stressed conditions.

Pandey *et al*. (1984) found that RLD increased in the lower soil profile of a peanut variety under drought conditions. Our results especially in Year 2 supported the previous findings although the results in Year 1 were rather confounding. However, Robertson *et al*. (1980) found no significant difference in rooting density of the peanut cultivar Florunner under irrigated and non-irrigated treatments. The contrasting results of previous studies were possibly due to the low number of peanut genotypes in their studies and differential responses of peanut genotypes to drought stress. Although a few peanut varieties were used in this study, they were selected in our experiment for contrasting levels of aflatoxin contamination (Girdthai *et al*., 2010a).

Root parameters such as RLD, rooting depth and root distribution have been established as constituting factors of drought resistance (Matsui and Singh, 2003). The ability of a plant to change its root distribution in the deeper soil profiles is an important mechanism for drought avoidance (Songsri *et al*., 2008; Benjamin and Nielsen, 2006).

Several researchers have been classified RLD as an indirect selection trait for improving drought adaptation of peanut (Songsri *et al*., 2008; Jongrungklang *et al.*, 2011, 2012). Similarly, RLD contributed to resistance to aflatoxin contamination under long-term drought (Arunyanark *et al*., 2009).

In this work, ICGV 98348 and ICGV 98324 had high root traits in the deeper soil layer (30-90 cm), high stomatal conductance and high water use efficiency (Koolachart *et al*., 2013), but they had low *A. flavus* colonization and low aflatoxin contamination. The relationships among these traits in these peanut varieties were consistent across years. However, some peanut varieties with high root traits did not show low *A. flavus* colonization and low aflatoxin contamination. These relationships seemed to be specific to some peanut varieties, and, therefore, selection of varieties with good and consistent values for these traits should reduce aflatoxin contamination under drought conditions. The varieties may use different strategies to maintain low aflatoxin contamination in drought conditions.

Our results indicated that % RLD, root surface area, root diameter and root volume in the deeper soil layer under terminal drought conditions contributed to reduce to aflatoxin contamination. The results revealed that peanut varieties with the ability to maintain high root traits in the deeper soil layer under stressed conditions are more resistant to aflatoxin contamination.

CONCLUSIONS

Peanut varieties with high root traits in the deeper soil layer and low *A. flavus* colonization could maintain low aflatoxin contamination. ICGV 98348

and ICGV 98324 were the best varieties for low aflatoxin contamination and low *A. flavus* colonization, and high root traits. Our studies demonstrated that root traits may be important traits related to aflatoxin contamination and confirmed that colonization is related to aflatoxin contamination. These findings indicated that roots traits may be useful alternative selection criteria for reduced aflatoxin contamination under terminal drought.

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