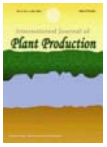

International Journal of Plant Production 6 (2), April 2012
ISSN: 1735-6814 (Print), 1735-8043 (Online)
www.ijpp.info

GUASNR

Association between aflatoxin contamination and N_2 fixation in peanut under drought conditions

A. Arunyanark[a], S. Pimratch[b], S. Jogloy[b,*], S. Wongkaew[c], N. Vorasoot[b], C. Akkasaeng[b], T. Kesmala[b], A. Patanothai[b], C.C. Holbrook[d]

[a]*Department of Agronomy, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Nakhon Pathom 73140, Thailand.*
[b]*Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand.*
[c]*School of Crop Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand.*
[d]*Crop Genetics and Breeding Research Unit, USDA/ARS, Coastal Plain Experiment Station, Tifton, Georgia 31793, USA.*
*Corresponding author. E-mail: sanun@kku.ac.th

Received 18 November 2011; Accepted after revision 20 January 2012; Published online 20 March 2012

Abstract

Traits related to nitrogen fixation may be used as indirect selection criteria for aflatoxin resistance in peanut. The aim of this study was to investigate the relationship between N_2 fixation traits and aflatoxin contamination in peanut under different drought conditions. Eleven peanut genotypes were evaluated under three water regimes for two seasons in the field. Data were observed on kernel infection by *Aspergillus flavus*, aflatoxin contamination, total nitrogen content, N_2 fixation and its related traits viz. nodule number, nodule dry weight and nitrogenese activity. Drought stress reduced total nitrogen content and N_2 fixation, but it increased kernel infection and aflatoxin contamination. Total nitrogen content, N_2 fixation and its related traits had negative and significant effects on kernel infection and aflatoxin contamination especially under drought conditions. In addition, negative correlations between kernel infection and aflatoxin contamination with drought tolerance index (DTI) of N_2 fixation traits were also found. The results indicated that the ability to maintain high N_2 fixation under drought conditions of peanut genotypes can result in better resistance to aflatoxin contamination.

Keywords: Aflatoxin contamination; *Aspergillus flavus*; Kernel infection; Drought tolerance; Nitrogen content; N_2 fixation.

Introduction

Aflatoxin contamination is the most important quality problem in peanuts throughout the world as it is related to serious health problems in human as well as in livestock. Alleviation of aflatoxin contamination through genetic manipulation has been a long-term goal of peanut breeders. Research has suggested that drought tolerance in peanut may have the potential to be used as an indirect selection criterion for resistance to pre-harvest aflatoxin contamination as drought tolerant lines generally display lower levels of pre-harvest aflatoxin contamination (Holbrook et al., 2000; Arunyanark et al., 2009; Arunyanark et al., 2010; Girdthai et al., 2010). Drought tolerance mechanisms can enhance the ability of genotypes to minimize aflatoxin production. Therefore, breeding of peanut for drought tolerance might be a promising strategy for improving resistance to aflatoxin contamination.

The ability of peanut to maintain high N_2 fixation under water limited conditions might be a mechanism of tolerance to drought stress (Pimratch et al., 2008b, Puangbut et al., 2011a). Atmospheric nitrogen can be assimilated into useful forms through symbiotic nitrogen fixation by legumes including peanut, in association with specific *Rhizobium* spp. or *Bradyrhizobium* spp. (Van Rossum et al., 1993; Giller, 2001). As nitrogen is an essential nutrient for growth and yield of peanut, genotypes with high nitrogen fixation would be expected to give higher yield under drought.

Pimratch et al. (2008b) and Puangbut et al. (2011b) reported that the ability to maintain high nitrogen fixation under drought stress could aid peanut genotypes in maintaining high yield under water limited conditions. Moreover, it has been well demonstrated in peanut that nitrogen fixation is closely related to nodule traits and nitrogenase activity and they have been used as surrogate traits for nitrogen fixation (Pimratch et al., 2008a).

Nitrogen fixation and its related traits may be used as indirect selection tools for aflatoxin resistance. The objective of this study was to investigate the relationship between N_2 fixation traits and aflatoxin contamination under different drought stress conditions.

Materials and Methods

Experimental design and treatments

The field experiment was conducted at the Field Crop Research Station, Khon Kaen University, Khon Kaen, Thailand during November 2003 to

March 2004, and October 2004 to February 2005. Soil type was a Yasothon series (Yt; fine-loamy; siliceous, isohypothermic, Oxic Paleustults). Analyses of soil in the field experiment showed a range of 0.030-0.035% for total nitrogen, 35.32-45.84 parts per million (ppm) for available phosphorus, 41.64-52.31 ppm for exchangeable potassium and 445.60-456.85 ppm for calcium. Clearly, N was inadequate but P, K and Ca were adequate (Marschner, 1995).

A split-plot in a randomized complete block design with four replications for two seasons was used. Three water regimes (field capacity (1 AW), 2/3 available water (AW) and 1/3 AW) were assigned in main plots and 11 peanut cultivars were arranged in sub-plots. Eleven peanut genotypes (ICGV 98300, ICGV 98303, ICGV 98305, ICGV 98308, ICGV 98324, ICGV 98330, ICGV 98348, ICGV 98353, Tifton-8, KK 60-3 and Tainan 9) were selected because of their reputed superior drought resistance characteristics. A non-nodulating line (Non-nod) obtained from ICRISAT was also included as a reference plant in determining nitrogen fixation (McDonagh et al., 1993).

Crop and water management

The crop was planted in eleven-row plots with 6 m length and spacing of 0.5 m between rows and of 0.2 m between plants within a row. Inoculation of rhizobium was accomplished by diluting commercial peat-based inoculums of *Bradyrhizobium* (mixture of strains THA 201 and THA 205; Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand) with water, and the slurry was spread on the rows of peanut soon after planting.

A subsoil-drip-irrigation system was installed to ensure a uniform supply of the correct amount of water to the plots. Soil moisture was initially maintained at field capacity until 14 days after emergence (DAE) in all treatments. Afterwards, stress treatments were initiated by withholding irrigation until the soil moisture levels was reduced to predetermined levels of 2/3 AW and 1/3 AW treatments, respectively. Water regime treatments were maintained at ±1% of the predetermined levels until harvest. In order to maintain the specified soil moisture regimes, calculated amounts of water were added to the respective main plots based on crop evapotranspiration, which were calculated as described by Doorenbos and Pruitt (1992) and Singh and Russell (1981).

The *A. flavus* isolated from a peanut field by the laboratory of Suranaree University of Technology, Nakhonratchasima province, Thailand, was used to increase the inoculum load in the soil. *A. flavus* inoculum was multiplied on peanut meal and inoculated at 30 DAE in the 2003/04 season and before planting in the 2004/05 season at the rate of 375 kg ha^{-1}.

Total nitrogen content, N_2 fixation and its related traits

Nodule number, nodule dry weight and nitrogenese activity were evaluated at 90 DAE (Pimratch et al., 2008a). Nitrogenase activity was evaluated by acetylene reduction assay (ARA) (Venkateswarlu et al., 1989) and read on a gas chromatography reader model GC-8A (Shimadzu Inc.). Ten plants in each plot were used as samples for nitrogenase activity determination, then the samples were washed in tap water and nodules were removed from roots, counted, oven dried (at 80 °C for 48 hours) and weighed.

For each plot, the above-ground biomasses at harvest were harvested from a ground area of 8 m^2. Fresh weights excluding roots were recorded in the field, and a randomly selected plant sample excluding root of two kgs was taken from each plot. Shoots, kernels and shells were separated and then oven-dried at 80 °C for 48 hours for nitrogen evaluation. Shoots, kernels and shells were separately ground using a hammer mill and a sub-sample of 0.3 g used for analysis. The nitrogen content was analysed by micro-Kjeldahl digestion. Total nitrogen was then determined using the automated indophenol method and read on a flow injection analyser model 5012 (Tecator inc.: Hoganas, Sweden). Fixed nitrogen was determined by N-difference method using the non-nodulating line as a reference plant. This method has been proven in previous studies to be as effective as ^{15}N Isotope dilution method in determining nitrogen fixation (McDonagh et al., 1993). Fixed nitrogen contents (shoot+kernel+shell) were calculated as:

Total fixed N_2=(Total N of each genotype)-(Total N of the non-nodulating line)

Percent of nitrogen derived from atmosphere (%Ndfa) was calculated as:

%Ndfa = ((Fixed nitrogen content) / (Total nitrogen content)) × 100

Partition of nitrogen to kernel was also calculated from the ratio of N of kernel to N of plant.

Drought tolerance index (DTI), as suggested by Nautiyal et al. (2002), was calculated for total nitrogen content, N_2 fixation and its related traits as

the ratio of each parameter in the stress treatment (1/3 available water) to the well-watered (1 available water) treatment.

Kernel infection by A. flavus and aflatoxin contamination

At harvest, two sets of 10 plants for each plot were randomly sampled. The first set was used to measure kernel infection and the second set was used to measure aflatoxin contamination. The pods were removed from the plants and sun-dried to < 9% moisture. One hundred mature pods were selected randomly and shelled. One hundred kernels were used for measuring *A. flavus* infection. The second set of ten plants was used for aflatoxin B_1 determination. A 100 g mature kernel sample was ground in an electric mill. For each assay, a 20 g sub-sample was used for aflatoxin extraction and then for aflatoxin analysis using a modified, direct competitive ELISA (Enzyme Linked Immunosorbent Assay) (Arunyanark et al., 2009).

Results

Effect of drought stress

Combined analysis of variance showed significant effects of seasons (S) on kernel infection by *A. flavus* ($P<0.01$) and aflatoxin contamination ($P<0.01$) (Table 1). The effects of water regimes (W) and genotypes (G) were also significant ($P<0.01$) for kernel infection, aflatoxin contamination, total nitrogen content, N_2 fixation and %Ndfa. Interaction effects were significant for all parameters. Generally, G×E interactions such as S×W, S×G, W×G, and S×W×G were highest for aflatoxin contamination.

Drought consistently increased kernel infection (Figure 1a) and aflatoxin contamination (Figure 1b) in both seasons. The well-watered treatment (1 AW) had lower kernel infection (10-33%) and aflatoxin contamination (7-13 ppb) (averaged over 11 genotypes) compared to 21-51% kernel infection and 29-75 ppb aflatoxin contamination in severe drought conditions (1/3 AW). Kernel infection and aflatoxin contamination were much higher in the 2004/05 season than in the 2003/04 season. In addition, genotype differences were observed for aflatoxin contamination (range 4 to 183 ppb) (data not shown) and kernel infection (range 6-68 %) especially under the severe drought treatments.

Table 1. Pooled analysis of variance over two seasons for kernel infection by *A. flavus*, aflatoxin contamination, total nitrogen content, N_2 fixation and percent of N derived from the atmosphere (%Ndfa).

	df	Mean squares									
		Kernel infection		Aflatoxin		Total nitrogen content		N_2 fixation		$\%N_2$ fixation	
Season (S)	1	52920	**	41712	**	0.026		0.006		7	
Rep. within season	6	877		687		0.180		0.341		1973	
Water regimes (W)	2	4708	**	38578	**	2.857	**	3.786	**	11806	**
S×W	2	552		9024	*	0.423	**	0.228		348	
Error A	12	240		1833		0.028		0.118		1162	
Genotypes (G)	10	859	**	11142	**	0.097	**	0.096	**	178	**
S×G	10	173	*	4612	**	0.021		0.024	*	23	
W×G	20	115		3303	**	0.028	**	0.030	**	66	**
S×W×G	20	59		1663	**	0.024	*	0.023	*	27	
Error B	180	81		631		0.013		0.012		26	

Significant at * P<0.05 and ** P<0.01 levels.

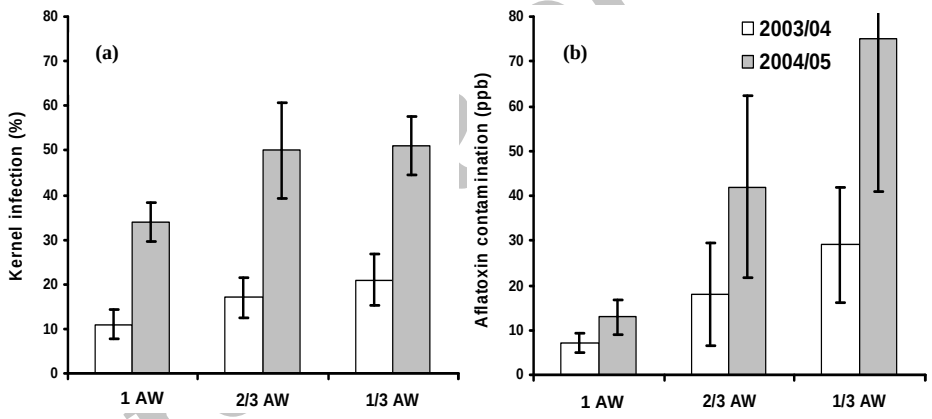

Figure 1. Kernel infection by *A. flavus* (a) and aflatoxin contamination (b) in different water regimes (1 available water (AW), 2/3 AW and 1/3 AW) in the 2003/04 and 2004/05 seasons. Error bars represent±SED.

There was a significant variation among peanut genotypes for total nitrogen content (range 0.49-1.20 g plant^{-1}) (Figure 2a), N_2 fixation (range 0.24-0.97 g plant^{-1}) (Figure 2b) and %Ndfa (range 46-81%) (Figure 2c). Drought stress consistently reduced total nitrogen content, N_2 fixation and %Ndfa. Moreover, highly significant and positive correlations between seasons were observed for total nitrogen content, N_2 fixation and %Ndfa

(r=0.77*, 0.83** and 0.90**) indicating that these characters were highly repeatable. The results suggest that it could be possible to select peanut genotypes with higher nitrogen content and N_2 fixation for a given location.

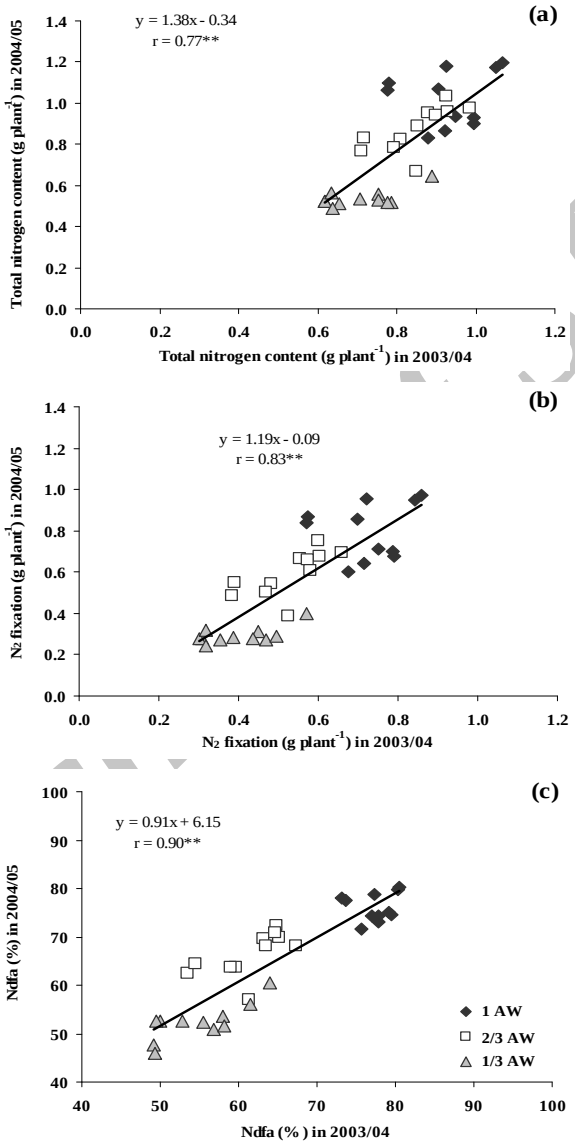

Figure 2. Pooled genotypic performance for total nitrogen content (a), N_2 fixation (b) and percent of N derived from atmosphere (Ndfa) (c) in different water regimes (1 available water (AW), 2/3 AW and 1/3 AW) in the 2003/04 and 2004/05 seasons. Significant at ** P<0.01 levels.

Relationships between kernel infection and aflatoxin contamination with N_2 fixation traits

There were negative and significant correlations between total nitrogen content and N_2 fixation with kernel infection and aflatoxin contamination when data were pooled across irrigation treatments (Table 2) in each season (r=-0.37* to -0.55**) and pooled data (r=-0.28* to -0.45**). The reverse relationships were also found between nodule number, nodule dry weight and ARA with kernel infection and aflatoxin contamination across water regimes in each season (r=-0.11 to -0.63**) and pooled data (r=-0.14 to -0.60**).

Table 2. Correlation coefficients between kernel infection and aflatoxin contamination with total nitrogen content, N_2 fixation and related traits of N_2 fixation across three water regimes.

	Kernel infection						Aflatoxin contamination					
	2003/04		2004/05		Pooled		2003/04		2004/05		Pooled	
	(n=33)		(n=33)		(n=66)		(n=33)		(n=33)		(n=66)	
Total nitrogen content	-0.42	*	-0.50	**	-0.32	**	-0.37	*	-0.48	**	-0.45	**
N_2 fixation	-0.50	**	-0.55	**	-0.28	*	-0.48	**	-0.49	**	-0.43	**
Nodule number	-0.32		-0.48	**	-0.14		-0.52	**	-0.50	**	-0.41	**
Nodule dry weight	-0.11		-0.51	**	-0.45	**	-0.35	*	-0.45	**	-0.47	**
ARA	-0.59	**	-0.51	**	-0.60	**	-0.63	**	-0.49	**	-0.50	**

Significant at * P<0.05 and ** P<0.01 levels. ARA, Acetylene reduction assay.

The correlations between kernel infection and aflatoxin contamination with nitrogen content, N_2 fixation and its related traits were evaluated across seasons under severe drought stress and non-stress conditions (Table 3). There were negative relationships between kernel infection and aflatoxin contamination with nitrogen content and N_2 fixation in the whole plant (shoot+shell+kernel) and shoot under drought conditions (r=-0.26 to -0.67**). However, such relationships with nitrogen content and N_2 fixation in kernels and nitrogen partitioning to kernels were positive (r=0.22 to 0.54**). The negative correlations in shoots cancelled out the positive correlations in kernels and resulted in weak correlations in the whole plant. Moreover, correlations between kernel infection and aflatoxin contamination with nodule dry weight and ARA under drought conditions were negative (r=-0.43* to -0.86**). In addition, the negative correlations between kernel infection and aflatoxin contamination with drought tolerance index (DTI) of

nitrogen content, N_2 fixation and its related traits were also found. In general, correlations between kernel infection and aflatoxin contamination with nitrogen traits were stronger under drought conditions than well-watered conditions.

Table 3. Correlation coefficients between kernel infection and aflatoxin contamination with total nitrogen content, N_2 fixation and related traits of N_2 fixation of 11 peanut genotypes in 1 available water (1 AW) and 1/3 available water (1/3 AW) across two seasons.

	Kernel infection						Aflatoxin contamination					
	1 AW		1/3 AW		DTI		1 AW		1/3 AW		DTI	
Total nitrogen content												
Plant (shoot+shell+kernel)	0.37		-0.67	**	-0.74	**	-0.09		-0.33		-0.28	
Shoot	-0.34		-0.62	**	-0.60	**	-0.41		-0.48	*	-0.56	**
Kernel	0.47	*	-0.07		-0.55	**	0.21		0.29		0.05	
Partitioning to kernel	0.41		0.34		-0.09		0.31		0.50	*	0.48	*
N_2 fixation												
Plant (shoot+shell+kernel)	0.30		-0.49	*	-0.61	**	-0.15		-0.26		-0.22	
Shoot	-0.43	*	-0.49	*	-0.34		-0.46	*	-0.44	*	-0.52	*
Kernel	0.49	*	0.22		-0.19		0.22		0.45	*	0.33	
Partitioning to kernel	0.44	*	0.43	*	0.20		0.35		0.54	**	0.61	**
Related traits of N_2 fixation												
Nodule number	0.73	**	-0.33		-0.58	**	0.40		-0.35		-0.41	
Nodule dry weight	0.43	*	-0.65	**	-0.81	**	0.16		-0.43	*	-0.50	*
ARA	-0.70	**	-0.86	**	0.13		-0.58	**	-0.60	**	0.11	

Significant at * P<0.05 and ** P<0.01 levels. ARA, Acetylene reduction assay.
Partitioning to kernel=N of kernel / N of plant;
DTI=drought tolerance index of nitrogen parameters was calculated by the ratio of stressed (1/3 available water) / non-stressed (1 available water) conditions.

Discussion

Drought is a major cause of aflatoxin contamination in peanut, and the more severe the drought conditions the greater the infection and contamination. Previous report showed that drought also severely reduced yield (Arunyanark et al., 2008).

Total nitrogen in the reference plant was lower than those in all nodulating plants (data not showed), indicating that the soil nitrogen was not sufficient, and growth of the test peanut lines depended largely on fixed nitrogen (Pimratch et al., 2008b). Our study suggested that symbiotic nitrogen fixation from the atmosphere was an important nitrogen source for normal productivity of peanut and accounted for 72-81% of total nitrogen

content under well-watered conditions. However, % nitrogen derived from the atmosphere was reduced to 46-64% under severe drought conditions.

Drought reduces fixed nitrogen content which in turn resulted in a decrease in total nitrogen content. Effects of drought stress on nitrogen fixation and nitrogen uptake depend on the degree of stress, the period of stress and the stage of crop development (Ahmadi et al., 2011; Giller, 2001; Tafteh and Sepaskhah, 2012). Moreover, drought stress also reduced traits related to nitrogen fixation such as nitrogenase activity, nodule number and nodule dry weight (Pimratch et al., 2008a).

Negative relationships between kernel infection and aflatoxin contamination with traits related to nitrogen fixation such as total nitrogen content, N_2 fixation nodule number, nodule dry weight and ARA were found in two seasons. Moreover, the correlations between kernel infection and aflatoxin contamination with nitrogen traits were stronger under drought conditions than well-watered conditions. The results indicated that the ability of genotypes to maintain high nitrogen content or N_2 fixation and its related traits under drought conditions may help to reduce kernel infection and aflatoxin contamination.

DTI for nitrogen traits might be used to explain how certain genotypes have higher performance in terms of total nitrogen content or N_2 fixation traits under drought. The negative relationships between kernel infection and aflatoxin contamination with DTI of nitrogen traits indicated that peanut genotypes which have a lower reduction for nitrogen traits also had low kernel infection and aflatoxin contamination when grown under drought conditions.

Earlier work showed that drought tolerance was related to aflatoxin contamination resistance. Holbrook et al. (2000) reported positive correlations between aflatoxin contamination with leaf temperature and visual stress ratings. More recently, drought tolerance traits such as harvest index, specific leaf area, relative water content, chlorophyll density, drought stress ratings and root length density were related with kernel infection and aflatoxin contamination under drought conditions (Arunyanark et al., 2009; Arunyanark et al., 2010; Girdthai et al., 2010). To the best of our knowledge, this is the first report showing evidence of the relationship between N_2 fixation traits and aflatoxin resistance in peanut.

However, nitrogen sources were also necessary for the fungal growth and aflatoxin biosynthesis (Luchese and Harrigan, 1993). Therefore, the correlation between kernel infection and aflatoxin contamination with nitrogen content and N_2 fixation were positive in kernel and nitrogen

partitioning to kernel. However, such relationships were negative in the whole plant and shoot under drought conditions, indicating the possibility to select peanut lines with high nitrogen content or N_2 fixation in the whole plant or shoot and low kernel infection and aflatoxin contamination in kernel under drought conditions.

Due to the strong relationships of nodule dry weight and ARA with aflatoxin resistance, these traits have high potential in selection for aflatoxin resistance. Nodule dry weight is simple to measure, and it may be practical and cost effective for application in breeding programs.

In conclusion, drought reduced total nitrogen content and N_2 fixation, but it increased kernel infection by *A. flavus* and aflatoxin contamination. This is the first report showing evidence that total nitrogen content, N_2 fixation and its related traits such as nodule number, nodule dry weight and ARA had negative and significant effects on kernel infection and aflatoxin contamination. Therefore, peanuts with the ability to maintain high nitrogen content or N_2 fixation and its related traits under drought conditions may also exhibit better aflatoxin resistance.

Acknowledgements

The authors are grateful for the financial support of the Royal Golden Jubilee Ph.D. Programme and Grateful acknowledgement is made to the Thailand Research Fund, the commission for High Education and Khon Kaen University for providing financial supports to this research through the Distinguish Research Professor Grant of Professor Dr. Aran Patanothai.

References

Ahmadi, S.H., Andersen, M.N., Laerke, P.E., Plauborg, F., Sepaskhah, A.R., Jensen, C.R., Hansen, S., 2011. Interaction effect of different irrigation strategies and soil textures on the nitrogen uptake of field grown potatoes. Int. J. Plant Prod. 5 (3), 263-274.

Arunyanark, A., Jogloy, S., Akkasaeng, C., Vorasoot, N., Kesmala, T., Nageswara Rao, R.C., Wright, G.C., Patanothai, A., 2008. Chlorophyll stability is an indicator of drought tolerance in peanut. J. Agron. Crop Sci. 194, 113-125.

Arunyanark, A., Jogloy, S., Akkasaeng, C., Vorasoot, N., Nageswara Rao, R.C., Wright, G.C., Patanothai, A., 2009. Association between aflatoxin contamination and drought tolerance traits in peanut. Field Crops Res. 114, 14-22.

Arunyanark, A., Jogloy, S., Akkasaeng, C., Vorasoot, N., Kesmala, T., Nageswara Rao, R.C., Wright, G.C., Patanothai, A., 2010. Heritability of aflatoxin resistance traits and correlation with drought tolerance traits in peanut. Field Crops Res. 117, 258-264.

Doorenbos, J., Pruitt, W.O., 1992. Calculation of crop water requirements. In: FAO Irrigation and Drainage, Paper No. 24. FAO of the United Nation. Rome, Italy, pp. l-65.

Giller, K.E., 2001. Nitrogen Fixation in Tropical Cropping Systems. 2nd edn. CAB International, Wallingford.

Girdthai, T., Jogloy, S., Vorasoot, N., Akkasaeng, C., Wongkaew, S., Holbrook, C.C., Patanothai, A., 2010. Associations between physiological traits for drought tolerance and aflatoxin contamination in peanut genotypes under terminal drought. Plant Breed. 129, 693-699.

Holbrook, C.C., Kvien, C.K., Rucker, K.S., Wilson, D.W., Hook, J.E., 2000. Preharvest aflatoxin contamination in drought tolerant and intolerant peanut genotypes. Peanut Sci. 27, 45-48.

Luchese, R.H., Harrigan, W.F., 1993. Biosynthesis of aflatoxin-the role of nutritional factors. J. Appl. Bacteriol. 74, 5-14.

Marschner, H., 1995. Mineral Nutrition of Higher Plants. 2nd edn. Academic Press, London.

McDonagh, J.F., Toomsan, B., Limpinantana, V., Giller, K.E., 1993. Estimates of the residual nitrogen benefit of groundnut to maize in Northeast Thailand. Plant and Soil, 154, 267-277.

Nautiyal, P.C., Nageswara Rao, R.C., Joshi, Y.C., 2002. Moisture-deficit-induced changes in leaf-water content, leaf carbon exchange rate and biomass production in groundnut cultivars differing in specific leaf area. Field Crop Res. 64, 67-79.

Pimratch, S., Jogloy, S., Vorasoot, N., Toomsan, B., Kesmala, T., Patanothai, A., Holbrook, C.C., 2008a. Effect of drought stress on traits releated to N_2 fixation in eleven peanut (*Arachis hypogaea* L.) genotypes differing in degrees of resistance to drought. Asian J. Plant Sci. 7 (4), 334-342.

Pimratch, S., Jogloy, S., Vorasoot, N., Toomsan, B., Patanothai, A., Holbrook, C.C., 2008b. Relationship between biomass production and nitrogen fixation under drought-stress conditions in peanut genotypes with different levels of drought resistance. J. Agron. Crop Sci. 194, 15-25.

Puangbut, D., Jogloy, S., Vorasoot, N., Akkasaeng, C., Patanothai. A., 2011a. Association of transpiration efficiency with N_2 fixation of peanut under early season drought. Int. J. Plant Prod. 5 (4), 381-394.

Puangbut, D., Jogloy, S., Kesmala, T., Vorasoot, N., Akkasaeng, C., Patanothai, A., Puppala, N., 2011b. Heritability of early season drought resistance traits and genotypic correlation of early season drought resistance and agronomic traits in peanut. SABRAO J. Breed. Genet. 43 (2), 165-187.

Singh, S.M., Russell, B., 1981. Water use by maize/pigeonpeas intercrop on a deep Vertisol. Proceedings of International Workshop on Pigeonpeas, ICRISAT Center, Patancheru, AP, India, 15-19 December 1980. 1, 271-282.

Tafteh, A., Sepaskhah, A.R., 2012. Yield and nitrogen leaching in maize field under different nitrogen rates and partial root drying irrigation. Int. J. Plant Prod. 6 (1), 93-114.

Van Rossum, D., Muyotcha, A., Verseveld, H.W.V., Stouthamer, A.H., Boogerd, F.C., 1993. Effects of *Bradyrhizobium* strain and host genotype, nodule dry weight and leaf area on groundnut (*Arachis hypogaea* L. ssp. *fastigiata*) yield. Plant and Soil, 154, 279-288.

Venkateswarlu, B., Maheswari, M., Saharan, N., 1989. Effects of water deficit on N_2 (C_2H_2) fixation in cowpea and groundnut. Plant and Soil, 114, 69-74.