References

Anderson, W. F., C. C. Holbrook, and D. M. Wilson. 1996. Development of greenhouse screening for resistance to *Aspergillus parasiticus* infection and preharvest aflatoxin contamination in peanut. Mycopathologia 135: 115-118.

Anderson, W. F., C. C. Holbrook, D. M. Wilson, and M. E. Matheron. 1995. Evaluation of preharvest aflatoxin contamination in several potentially resistant peanut genotypes. Peanut Science 22: 29-32.

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

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

Blankenship, P.D., R.J. Cole, T.H. Sanders, and R.A. Hill. 1984. Effect of geocarposphere temperature on pre-harvest colonization of drought stressed peanuts by *Aspergillus flavus* and subsequent aflatoxin concentration. Mycopathologia 85: 69–74.

Boote, K. J. 1982. Growth stage of peanut (*Arachis hypogaea* L.). Peanut Science 9: 35–40.

Chenault, K.D., H.A. Melouk, and C.C. Holbrook. 2004. Post-harvest aflatoxin accumulation in transgenic peanut lines containing anti-fungal genes. Phytopathology 94: S18.

Clavel, D, O. Diouf, J. L. Khalfaoui, and S. Braconnier. 2006. Genotypes variations in fluorescence parameters among closely related groundnut (*Arachis hypogaea* L.) lines and their potential for drought screening programs. Crop Science 34: 92–97.

Coffelt, T. A., R. O. Hammons, W. D. Branch, R. W. Mozingo, P. M. Phipps, J. C. Smith, R. E. Lynch, C. S. Kvien, D. L. Ketring, D. M. Porter, and A. C. Mixon. 1985. Registration of Tifton-8 peanut germplasm. Crop Science 25: 203.

Cole, R. J., V. S. Sobolev, and J. W. Dorner. 1993. Potentially important sources of resistance to prevention of preharvest aflatoxin contamination in peanuts. Vol. 25 pp. 78. *In* Proceedings in American Peanut Research and Education Society, Huntsville, Alabama, USA.

Del Rosario, D.A., and F.F. Fajado. 1988. Morphophysiological responses of ten peanut (*Arachis hypogaea* L.) varieties to drought stress. The Philippine Agriculturist 71: 447-459.

Doorenbos, J., and W.O. Pruitt. 1992. Calculation of crop water requirements, pp. 1–65. *In* FAO Irrigation and Drainage Paper No: 24. FAO of the United Nation. Rome, Italy.

Dorner, J. W., R.J. Cole, T.H. Sanders, and P.D. Blankenship. 1989. Interrelationship of kernel water activity, soil-temperature, maturity, and phytoalexin production in preharvest aflatoxin contamination of drought-stressed peanuts. Mycopathologia 105: 117-128.

Gomez, K.A., and A.A. Gomez. 1984. Statistical procedures for agricultural research. 2nd ed. John Wiley & Sons: New York.

Holbrook, C. C., B. Z. Guo, D. M. Wilson, and P. Timper. 2009. The U.S. breeding program to develop peanut with drought tolerance and reduced aflatoxin contamination. Peanut Science 36: 50-53.

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

Holbrook, C. C., D. M. Wilson, M. E. Matheron, J. E. Hunter, D. A., Knauft, and D. W. Gorbet. 2000b. *Aspergillus* colonization and aflatoxin contamination in peanut genotypes with reduced linoleic acid composition. Plant Disease 84: 148-150.

Holbrook, C.C., M. E. Matheron, D.W.. Wilson, W.F. Anderson, M. E. Will, and A. J. Noden. 1994. Development of a large-scale field screening system for resistance to preharvest aflatoxin contamination. Peanut Science 21: 20-22.

Holbrook, C. C., P. Ozias-Akins, P. Timper, D. M. Wilson, E. Cantonwine, B. Z. Guo, D. G. Sullivan, and W. Dong. 2008. Research from the coastal plain experiment station, Tifton, Georgia to minimize aflatoxin contamination in peanut. Toxin Reviews 27: 391-410.

Klich, M.A., L.H. Tiffany, and G. Knaphus. 1992. Ecology of the *Aspergilli* of soils and litter, pp. 329–354. *In* J.W. Bennett, and M.A. Klich (eds.), *Aspergillus*: biology and industrial applications. Butterworth Heineman: Boston.

Kramer, P. J. 1980. Drought stress and the origin of adaptation. pp. 7–20 *In* N. C. Turner, and P. J. Kramer, (eds.), Adaptation of Plant to Water and High Temperature Stress, John Wiley & Sons, New York.

Moran, R. 1981. Formulas for determination of chlorophyll pigment extracted with N,N-Dimethyl formamide. Plant Physiology 69: 1376-1381.

Nageswara Rao, R.C., and G.C. Wright. 1994. Stability of the relationship between specific leaf area and carbon isotope discrimination across environments in peanut. Crop Science 34: 98–103.

Nageswara Rao, R.C., H.S. Talwar, and G.C. Wright. 2001. Rapid assessment of specific leaf area and leaf N in peanut (*Arachis hypogaea.* L) using chlorophyll meter. Journal of Agronomy and Crop Science 186: 175–182.

Nageswara Rao, R. C., L. J. Reddy, V. K. Mehan, S. N. Nigam and D. McDonald. 1992. Drought research on groundnut at ICRITSAT. pp. 455. *In* N. S. Nigam, (ed.) Proceedings of an International Workshop, Groundnut-a Global Perspective, ICRISAT center, Patancheru, Andhra Pradesh, India.

Nageswara Rao, R.C., M. Udayakumar, G.D. Farquhar, H.S. Talwar, and T.G. Prasad. 1995. Variation in carbon isotope discrimination and its relationship to specific leaf area and ribulose-1, 5-bisphosphate carboxylase content in groundnut genotypes. Australian Journal of Plant Physiology 22: 545–551.

Nautiyal, P. C., R. C. Nageswara Rao, and Y. C. Joshi. 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 Crops Research 74: 67–79.

Nigam S.N., M.S. Basu, and A.W. Cruickshank. 2003. Hybridization and description of the trait–based and empirical selection programs, pp. 15–17. *In* A.W. Cruickshank, N.C. Rachaputi, G.C. Wright, and S.N. Nigam (eds.), Breeding for drought–resistant peanuts. 25–27 February 2002. ICRISAT Centre. Patancheru, Andhra Pradesh, India.

Nigam, S. N., S. Chandra, K. R. Sridevi, M. Bhukta, A. G. S. Reddy, N. R. Rachaputi, G. C. Wright, P. V. Reddy, M. P. Deshmukh, R. K. Mathur, M. S. Basus, S. Vasundhara, P. V. Varman and A. K. Nagda. 2005. Efficiency of physiological trait-based and empirical selection approaches for drought tolerance in groundnut. Annals of Applied Biology 146: 433–439.

Rucker, K. S., C. J. Kvien, C. C., Holbrook, and J. E. Hook. 1995. Identification of peanut genotypes with improved drought avoidance traits. Peanut Science 22: 14-18.

SAS Institute. 1990. SAS/STAT 6.12; SAS/STAT User's Guide. SAS Institute, Cary, North Carolina.

Singh, S., and M.B. Russel. 1981. Water use by maize/pigeonpea intercrop on a deep Vertisol, Vol. 1, pp. 271–282. *In* Proceedings of International workshop on pigeonpeas. 15–19 December 1980. ICRISAT Center. Patancheru, Andhra Pradesh, India.

Sobolev, V., B. Guo, C.C., Holbrook, and R.E. Lynch. 2007. Interrelationship of phytoalexin production and disease resistance in selected peanut genotypes. Journal of Agricultural and Food Chemistry 55: 2195-2200.

Vorasoot, N., P. Songsri, C. Akkasaeng, S. Jogloy, and A. Patanothai. 2003. Effect of water stress on yield and agronomic characters of peanut (*Arachis hypogaea* L.). Songklanakarin Journal of Science and Technology 25: 283–288.

Wotton, H. R., and R. N. Strange. 1985. Circumstantial evidence for phytoalexin involvement in the resistance of peanuts to *Aspergillus flavus*. Journal of General Microbiology 131: 487-494.

Wright, G.C., and R.C. Nageswara Rao. 1994. Groundnut water relations. pp. 281–325. *In* J. Smartt (ed.) The Groundnut Crop. A Scientific Basis for Improvement. Chapman & Hall, London.

Wright, G.C., K.T. Hubick, and G.D. Farquhar. 1988. Discrimination in carbon isotope of leaves correlates with water–use efficiency of field grown peanut cultivars. Australian Journal of Plant Physiology 15: 815-825.

Wright, G.C., R.C. Nageswara Rao, and G.D. Farquhar. 1994. Water use efficiency and carbon isotope discrimination in peanut under water deficit conditions. Crop Science 34: 92–97.

CHAPTER V

HERITABILITY ESTIMATES OF THE PHYSIOLOGICAL TRAITS FOR DROUGHT TOLERANCE AND GENOTYPIC AND PHENOTYPIC CORRELATIONS WITH AGRONOMIC TRAITS IN PEANUT (*Arachis hypogaea* L.) UNDER TERMINAL DROUGHT CONDITIONS

Introduction

Improvement of drought tolerance in peanut (*Arachis hypogaea* L.), an important oil and cash crop, would be beneficial in rainfed regions where drought is a major constraint limiting productivity and quality. Terminal drought occurring during the seed filling phase of peanut (Boote, 1982) has been observed to decrease pod yield and increase preharvest aflatoxin contamination (Dorner et al., 1989; Nageswara Rao et al., 1985; Ndunguru et al., 1995; Ravindra et al., 1990; Wright et al., 1991). Breeding peanut varieties with drought resistance is seen as providing an importance and sustainable part of the solution. In addition, preharvest aflatoxin contamination may be reduced with improved resistance to drought (Cole et al., 1993; Girdthai et al., 2008; Holbrook et al., 2008; 2009). Holbrook et al. (2000) and Girdthai et al. (2008) found that some drought resistant genotypes were observed to have lower aflatoxin contamination. However, breeding progress for drought tolerance in peanut based on selection for yield only have been slow due to large and uncontrollable genotype by environment (G x E) interactions. Breeding approaches using physiological traits having high heritability and low G x E interactions can improve selection efficiency for superior drought-tolerant genotypes, and supplement the selection based on yield (Blum, 1988; Falconer and Mackay, 1996).

This paper was submitted for publication in Field Crops Research.

Putative selection criteria that could be used as indirect selection to increase drought tolerance in peanut have been identified (Craufurd et al., 1999; Hubick et al., 1986; Nigam et al., 2005; Wright et al., 1988; 1994; Wright and Nageswara Rao, 1994). Wallace et al. (1993) suggested that indirect selection for yield will be most effective when applied to traits that already integrate most of the genetic and environmental effects that lead to yield. Passioura (1986) have proposed a simple model of yield based on the facts that pod yield is a function of water transpiration (T), water used efficiency (WUE), and harvest index (HI). Drought tolerance might be enhanced by improvement of soil water extraction capability or improvements in WUE, or integration of both (Wright and Nageswara Rao, 1994; Hebbar et al., 1994). Improvement of WUE could potentially lead to increased yield under limited moisture availability. However, WUE is not easy to measure and may not be feasible selection criteria in large segregating breeding populations. Wright et al. (1988) and Wright et al. (1994) have found WUE to be negatively correlated with carbon isotope discrimination (Δ) and specific leaf area (SLA) over wide ranges of varieties and environments, but analysis of Δ are expensive and not feasible everywhere. SLA which is negatively related to leaf thickness and photosynthetic capacity can be measured easily and inexpensively. Although SLA is affected by environment and genotype, the relationship between SLA and Δ is apparently stable across environments in peanut (Nageswara Rao and Wright, 1994). This confirmed that SLA can be used as a surrogate trait to increase WUE in peanut. Nageswara Rao et al. (2001) and Upadyaya (2005) found a significant negative correlation between the SPAD chlorophyll meter reading (SCMR), a rapid assessment for drought tolerance in peanut and SLA, and suggested that this chlorophyll meter could be used as a rapid and reliable measure to identify genotypes with low SLA and hence high transpiration efficiency (TE) in peanut. Harvest index (HI) is an important trait that provides a measure of total biomass actually partitioned into pod yield. Genotypic correlations between HI and SLA and SCMR were also found in peanut under well-watered and drought conditions (Songsri et al., 2008). Duncan et al. (1978) suggested that partitioning of assimilates expressed as HI has considerable effects on pod yield, and breeding for high pod yield might be accomplished by selection for high HI.

The effectiveness of selection for a trait depends on the relative magnitudes of the genetic and non genetic causes expressed as the heritability of the trait. Relatively few studies to date have investigated the heritabilities and genotypic correlations of physiological traits for drought resistance in peanut, and none have been done under terminal drought conditions. Hubick et al. (1988) reported that heritability estimates were high for TE and especially for Δ, and there was no significant G x E interaction for Δ. Songsri et al., (2008) found that heritabilities of physiological traits for drought resistance in peanut were high ($h^2 > 0.50$) under drought and well-watered conditions, and physiological traits like SLA, SCMR, HI, and drought tolerance index of pod yield and biomass were associated well with agronomic traits under long periods of drought. Cruickshank et al. (2004) also found that broad sense heritability estimates for HI was high under rainfed conditions. However, they did not focus on terminal drought which is the most important period affecting yield and inducing preharvest aflatoxin contamination.

Efficient utilization of the physiological traits for improving drought resistance in a breeding program requires an understanding of the inheritance and genetic relationships of the trait that is available for selection. Limited information is available on heritability and genotypic associations of physiological traits linked to yield and agronomic traits in peanut. Hence, the objectives of this study were to estimate the heritabilities of terminal drought resistance traits, genotypic and phenotypic correlations between drought resistance traits and agronomic traits in peanut under terminal drought, and relationships between drought resistance traits under well-watered and terminal drought conditions in order to predict indirect responses to selection for drought resistance.

Materials and methods

Genetics materials and experimental design

Four peanut F_1 hybrids (ICGV 98348 x Tainan 9, ICGV 98348 x KK60-3, ICGV 98353 x Tainan 9 and ICGV 98353 x KK60-3) were generated from the hybridization of 2 drought-resistant lines (ICGV 98348 and ICGV 98353; medium maturing (110 days to maturity) and medium seeded type) selected for low yield reduction and high pod yield under well-watered and terminal drought conditions with KK60-3 (late maturing (120 days to maturity) and large seeded type) selected for high biomass and Tainan 9 (early maturing (100 days to maturity) and medium seeded type) having low seed yield and biomass under drought. KK60-3, ICGV 98348, and ICGV 98353 are know to have high SCMR and low SLA under stress conditions, Tainan 9 has high SLA and low SCMR under both stressed and non-stressed conditions. The F_1 seeds were planted and their seeds harvested in bulk for each cross. In F_2 and F_3 generations, one pod was kept from each plant and bulked for each cross. Line separation was carried out in the F_4 generation. A total of 140 lines (35 lines for each cross) were randomly selected and multiplied in the F_5 generation.

Parental lines and the 140 families from 4 crosses were evaluated in the $F_{4:6}$ and $F_{4:7}$ generations (F_4 – derived lines in the F_6 and F_7 generations, respectively) under two soil moisture levels (field capacity (FC) and 1/3 available soil water (1/3 AW) at 80 days after planting (DAP) to final harvest) for two years in dry season 2006/07 and repeated in dry season 2007/08. A split plot design with four replications was used for both years at the Field Crop Research Station, Faculty of Agriculture Khon Kaen University located in Khon Kaen Province, Thailand (latitude 16° 28′ N, longitude 102° 48′ E, 200 m above sea level). Soil type is Yasothon Series (loamy sand, Ocix Paleustults) with the soil moisture of FC is 10.2 % and permanent wilting point is 3.1 %. Two soil moisture levels, FC (10.2 %) and 1/3 AW (5.5 %) in 0-60 cm depth were assigned as main plots, and peanut lines were laid out in subplots. Each entry was planted in five row plots with 3 m length. Spacing was 40 cm between rows and 20 cm between plants within the row.

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 large seeded genotypes were treated with ethrel (2-chloroethylphospphonic acid) 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 14 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 40 cm between drip lines at 10 cm below the soil surface midway between peanut rows to supply water to the crop and fitted with a pressure valve and a water meter to ensure a uniform supply of the required amounts of water. Soil water level was maintained at FC at 0-60 cm depth. This soil depth should reasonably cover the majority of the rooting zone. In 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 each plot. 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.

Data collection

Weather parameters

Relative humidity, pan evaporation, rainfall, maximum and minimum air temperature, and solar radiation during two cropping seasons were recorded daily from sowing until final harvest by a meteorological station located 600 m away from the experimental field. Forty mm of the total amount of rainfall was recorded during 80-100 DAP in 2006/07, and 22.7 mm was recorded during this period in 2007/08 (Figure 1). Air temperature, relative humidity and evaporation in 2006/07 were higher than in the 2007/08, especially during the water stress period. During stress period (80 DAP to final harvest), mean evaporation was 6.0 and 5.0 mm in 2006/07 and 2007/08, respectively. The maximum and minimum air temperature ranged from 11.8 to 38.5 °C in 2006/07 and 14.5 to 35.2 °C in 2007/08, being lower during 80–110 DAP in 2007/08. Relative humidity ranged from 54 to 93 % in 2006/07 and from 57 to 92 % in 2007/08. The seasonal mean solar radiation was 0.13 and 0.11 Cal cm^{-2} in 2006/07 and 2007/08, respectively.

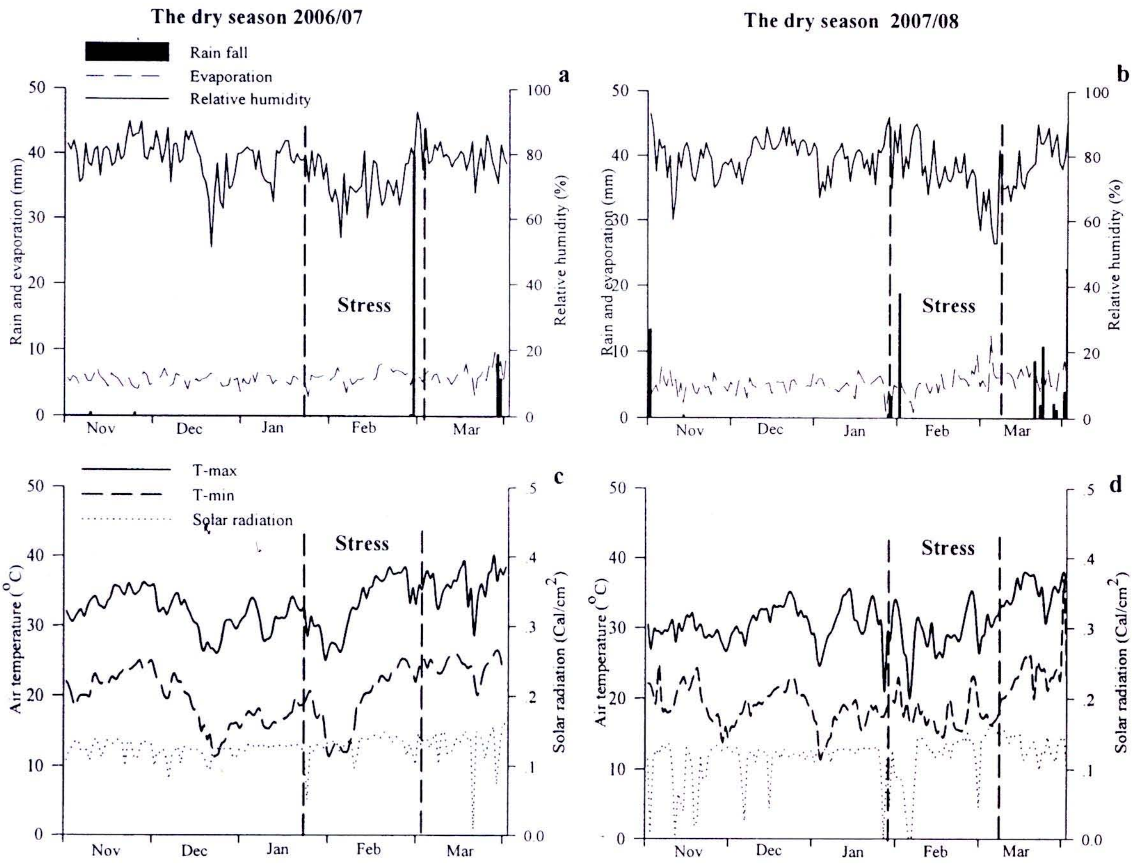

Figure 1 Relative humidity (%) (a and b), pan evaporation (mm) (a and b), rainfall (mm) (a and b), maximum and minimum air temperature (°C) (c and d), and solar radiation (Cal/cm^2) (c and d) during the crop growth period in 2006/07 (a and c) and in 2007/08 (c and d).

Soil moisture status

Soil moisture in each main 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, and 55-60 cm. Readings were taken at two positions in each main plot. The measurement before planting was used for calculating the correct amount of water to be applied for the crop. Soil moisture volume fraction was also monitored at 10 day intervals from planting to final harvest using a neutron moisture meter (Type I.H. II SER, no. N0152, Ambe Didcot Instruments Co. Ltd, Abingdon, UK). Five aluminium access tubes were installed in each main plot. Readings were taken in access tubes from the depth of 30-90 cm at 30 cm intervals.

SPAD chlorophyll meter reading and specific leaf area

Data were recorded for SCMR and SLA at 80 DAP. Five plants were randomly selected in each plot to record SCMR and SLA following the procedure described by Nageswara Rao et al. (2001). The second fully expanded leaves were detached from the chosen plants at 10 AM and brought to the laboratory in zipped polythene bags for recording observations. SCMR was recorded using a Minolta SPAD-502 meter (Minolta SPAD-meter, Tokyo, Japan) on the four leaflets from each leaf. An average SCMR for each plot was derived from 20 single observations (four leaflets x 5 plants plot^{-1}). In recording the SCMR, care was taken to ensure that the SPAD meter sensor fully covered the leaf lamina and that interference from veins and midribs was avoided.

After recording SCMR, the leaf area of all five sampled plants was measured with a leaf area meter (LI 3100C Area Meter, LI-COR Inc., USA) after which the leaves were dried in an oven at 80°C for at least 48 hours to determine leaf dry weight. Immediately after drying, the leaves were weighed and the SLA was derived as leaf area per unit leaf dry weight (cm^2 g^{-1}).

Agronomic traits

For each plot excluding boarder plants, three rows with 2.6 m in length (3.12 m^2) were harvested at maturity (R8) (Boote, 1982), and their pods and roots were removed before taking fresh shoot weight in the field. Five plants were randomly selected for measuring shoot fresh weight and then oven dried at 80°C for at least 48 hours and dry weight was measured. Shoot dry matter was then calculated and used in determining shoot dry weight for a plot. Pod yields were weighed after air drying to approximately 7-8 % moisture content. The number of mature pods per plant (mature pods were separated from immature pods, which were identified by dark internal pericarp color), number of seeds per pod, and 100 seed weight were also recorded at final harvest. HI was computed by the following formula:

HI = pod weight / total biomass

Statistical analysis

Analysis of variance was performed for each trait in each year following a split plot design (Gomez and Gomez, 1984). Calculation procedures were conducted using the MSTAT-C package (Bricker, 1989). Because water regime x genotype interaction was significant, each water regime was analyzed separately according to a randomized complete block design (RCBD) (Gomez and Gomez, 1984).

Estimates of broad sense heritability for the four crosses were calculated by partitioning variance components of family mean squares to pooled environmental variance (δ^2_E) and genotypic variance (δ^2_G), and then broad sense heritability estimates (h^2_b) were calculated as follows (Holland et al., 2003):

$$h^2_b = \delta^2_G / \delta^2_P$$

$$\delta^2_P = \delta^2_G + \delta^2_{GE} /e + \delta^2_E /re$$

where, h^2_b = broad sense heritability, δ^2_G = genotypic variation, δ^2_P = phenotypic variation, r = number of replications, and e = number of environments. The standard error (SE) of heritability (Singh et al., 1993) for each trait was calculated to give a measure of the precision of the estimate.

As the evaluation of heritability was conducted in late generations (F_6 and F_7) of segregating materials when most genes were nearly fixed in individual genotypes, it would be expected that additive genetic variances for the traits under study were fixed through generation advance (Holland, 2001).

Phenotypic and genotypic correlations between drought tolerance traits and agronomic traits, and correlations among physiological traits were calculated following the methods of Falconer and Mackay (1996) as follows (Table 1):

Phenotypic correlation, $r_P = (M_3 * M_3) / [(M_3 * M_3)]^{1/2}$

Genotypic correlation, $r_G = (M_3 * M_3 - M_2 * M_2) / [(M_3 * - M *_2) (M_3 - M_2)]^{1/2}$

Simple correlations were used to determine the relationships between biomass, pod yield, and drought resistance traits under well-watered and drought conditions to understand whether the performance of peanut genotypes were consistent across environments.

Table 1 Analysis of variance of cross and cross product.

Source of variation	Degrees of freedom	Mean square of character X	Y	MCP[†]	EMS[‡]	EMCP[§]
Year (Y)	Y-1					
Rep. within Y	Y(r-1)					
Families (F)	F-1	M_3*	M_3	$M^*_3 M_3$	$\delta^2_E + r\delta^2_{FE} + re\delta^2_F$	$\delta_{E*E}+r\delta_{FE*FE}+re\delta_{F*F}$
F x Y	(F-l)(Y-1)	M_2*	M_2	$M^*_2 M_2$	$\delta^2_E + r\delta^2_{FE}$	$\delta_{E*E}+ r\delta_{FE*FE}$
Pooled error	Y(r-1)(F-1)	M_1*	M_1	$M^*_1 M_1$	δ^2_E	δ_{E*E}

[†]MCP, mean square of cross product

[‡]EMS, expected mean square

[§]EMCP, expected mean square of cross product

Results and Discussions

Soil moisture data

Soil moisture data between water stress treatments were different in both years. Soil moisture measured by Neutron probe agreed well with those measured by Gravimetric method. Average soil moisture under the drought conditions at 80 DAP (5.7 % in both years) were less than the non-stressed treatment (11.5 % in 2006/07 and 10.2 % in 2007/08, respectively) (Figure 2). Under drought treatment, mean soil moisture during the growing seasons was 8.2 % and 8.1 % in 2004/05 and 2005/06, respectively. Soil moisture under drought conditions slightly decreased from 60 DAP to 80 DAP. Soil moisture under the stressed treatment during the end of the season (80-120 DAP) were 5.7 to 5.9 and 5.7 to 5.2 in 2007/08 and 2008/09, respectively. After 80 DAP, the soil moisture content of both treatments was held fairly constant until harvest. These results confirmed the soil moisture data in indicating that the degrees of drought were reasonably controlled at the predetermined levels.

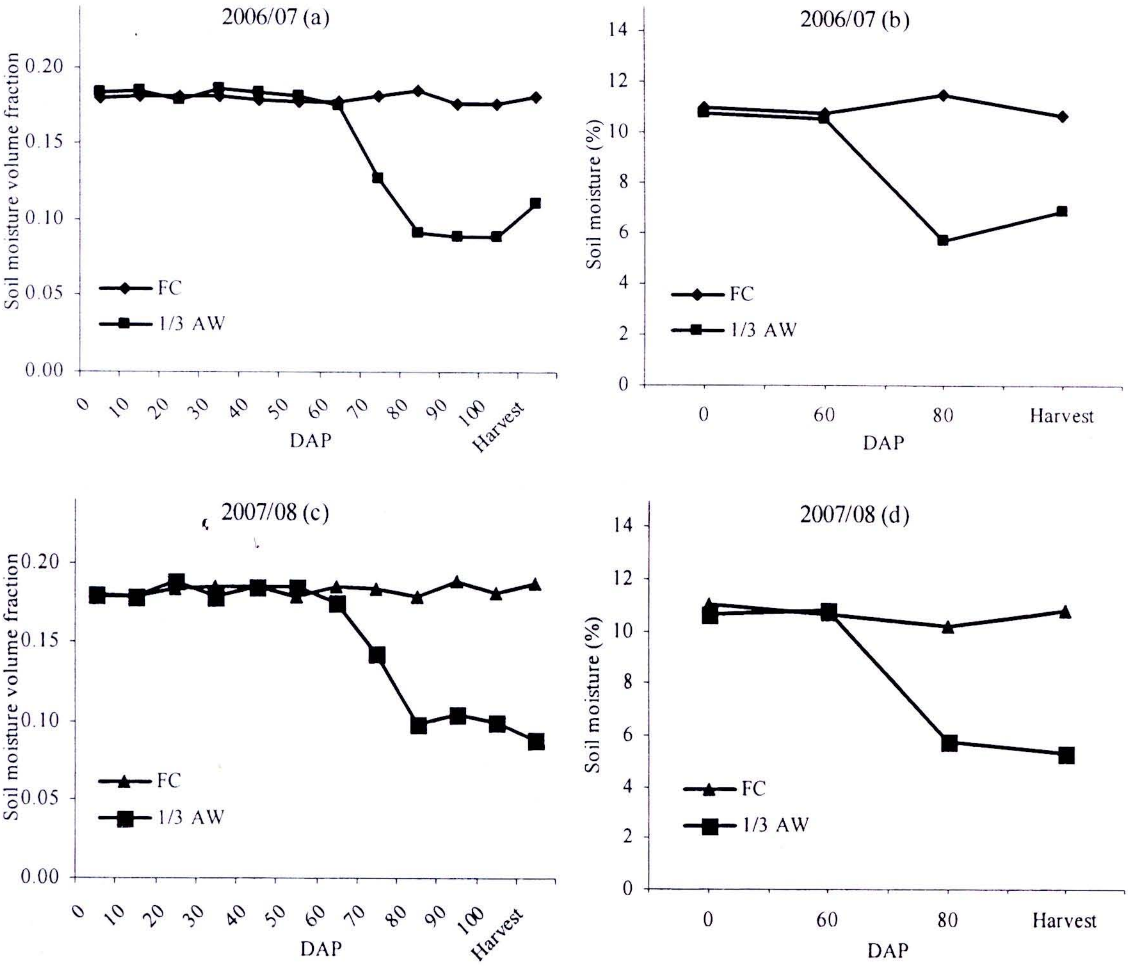

Figure 2 Soil moisture volume fraction (a and c) at planting, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 days after planting (DAP), and at final harvest and gravimetric soil moisture content (b and d) at planting, 60, 80 DAP and at final harvest under different water regimes [field capacity (FC) and 1/3 available water (1/3 AW)] average from 0-60 cm depth in 20062007 (a and b) and 2007/2008 (c and d).

Combine analysis

Combine analysis of variance showed large and significant differences between all 140 genotypes for all traits ($P \leq 0.01$) (Table 2). This reveals that the tested progeny displayed high variation. Hence, heritability of the traits can be estimated in these populations. Significant difference in years for HI, SLA under both stressed and non-stressed conditions and SCMR under non-stressed were also found ($P \leq 0.05$ to $P \leq 0.01$), but were not found for pod yield and biomass. Differences among interaction effects of year x genotypes (Y x G) for pod yield, biomass, HI under stressed and non-stressed conditions, and SLA under stressed were also significant ($P \leq 0.05$ to $P \leq 0.01$). Y x G interaction effects for SCMR and SLA were lower than PY and BIO. The Y x G interaction effect was not significant for SCMR under both water regimes and SLA under non-stressed conditions. The significant G x E interaction indicates that relative performance across environments is inconsistent among genotypes. For traits to be useful in breeding programs, they must be consistent from year to year. In this study, SCMR, and SLA showed a high degree of consistency in comparison to yield and biomass and thus it is appropriate to use them for screening peanut with drought resistance.

Table 2 Mean squares from the combined ANOVA for pod yield, biomass, and harvest index (HI) at final harvest and the physiological traits [SPAD chlorophyll meter reading (SCMR) and specific leaf area (SLA)] at 80 days after planting under field capacity (FC) and 1/3 available water (AW) of 140 genotypes in the dry season of 2006/07 and 2007/08.

Source of		Pod yield (kg ha^{-1})		Biomass (kg ha^{-1})		HI	
variation	DF	FC	1/3AW	FC	1/3AW	FC	1/3AW
Year (Y)	1	1293156	8936073	15150000	5852251	0.131*	0.301*
Rep. within Y	6	5027895	9553543	46850000	31130000	0.020	0.050
Genotypes (G)	139	2981814**	2407581**	21990000**	16050000**	0.025**	0.022**
Y x G	139	931405**	555736**	6699913**	4920515**	0.007**	0.005**
Pooled error	834	386108	313656	3166971	2143356	0.003	0.003

Source of		SCMR		SLA (cm^2g^{-1})	
variation	DF	FC	1/3AW	FC	1/3AW
Year (Y)	1	1092**	310	20264*	522936**
Rep. within Y	6	50	69	3817	3574
Genotypes (G)	139	48**	52**	775**	1207**
Y x G	139	6	9	187	384*
Pooled error	834	8	10	233	311

* and ** significant at $P \leq 0.05$ and significant at $P \leq 0.01$, respectively.

Heritability of traits

Heritability is a function of a breeding population and the conditions under which a study is conducted (Falconer and Mackay, 1996). It provides an indication of the expected response to selection in a segregating population, and is useful in designing an effective breeding strategy. In this study, heritability estimates for physiological traits were higher than for agronomic traits, and varied among crosses (Table 3). The heritabilities for pod yield (ranged from 0.25 to 0.79) and biomass (ranged from 0.17 to 0.66) were moderate, but high for HI (ranged from 0.58 to 0.85), SCMR (ranged from 0.66 to 0.91), and SLA (ranged from 0.64 to 0.90). The estimates of high heritability for physiological traits in the present study were generally in agreement with those previously reported by Songsri et al., (2008). Ntare and Williams (1998) also reported that heritability of pod yield was lower than partitioning coefficient but higher than other physiological components (crop growth rate and duration of reproduction growth) of their yield model. Cruickshank et al. (2004) also found that heritability estimates for HI were high (varied from 58-85 %) and varied significantly between crosses depending on levels of genetic variation in parents. In the present, the heritabilities for all three physiological traits ranged from 0.58 to 0.91, and the heritabilities for pod yield and biomass ranged from 0.17 to 0.79. Standard errors for physiological traits were also lower than for pod yield and biomass, especially under non-stressed conditions. Thus, the expected genetic gain per cycle of selection will be less for pod yield and biomass compared with HI, SCMR, and SLA. The large heritability for HI and for SCMR and SLA indicates that selection for these traits should be very effective. Heritabilities for traits were similar under different water regime and positive correlations between traits under different water regimes were significant (r = 0.32**-0.44**) (Table 3), indicating that these traits could be selected under either well-watered or terminal drought conditions.

Table 3 Broad sense heritability estimates for pod yield, biomass, harvest index (HI), SPAD chlorophyll meter reading (SCMR), and specific leaf area (SLA) and correlation between these traits under well-watered conditions and drought conditions of four crosses of peanut in the dry season of 2006/07 and 2007/08.

Peanut cross	Broad sense heritability				
	Pod yield	Biomass	HI	SCMR	SLA
Well-watered conditions					
ICGV 98348 x Tainan 9	0.43 ± 0.32‡	0.65 ± 0.24	0.67 ± 0.23	0.87 ± 0.13	0.84 ± 0.16
ICGV 98348 x KK 60-3	0.73 ± 0.20	0.52 ± 0.29	0.77 ± 0.18	0.86 ± 0.14	0.75 ± 0.20
ICGV 98353 x Tainan 9	0.60 ± 0.26	0.49 ± 0.30	0.65 ± 0.25	0.91 ± 0.10	0.73 ± 0.21
ICGV 98353 x KK 60-3	0.25 ± 0.37	0.17 ± 0.37	0.74 ± 0.20	0.66 ± 0.23	0.79 ± 0.18
Drought conditions					
ICGV 98348 x Tainan 9	0.57 ± 0.27	0.53 ± 0.29	0.58 ± 0.27	0.76 ± 0.19	0.64 ± 0.24
ICGV 98348 x KK 60-3	0.75 ± 0.19	0.66 ± 0.23	0.85 ± 0.14	0.71 ± 0.21	0.90 ± 0.10
ICGV 98353 x Tainan 9	0.79 ± 0.17	0.32 ± 0.34	0.79 ± 0.17	0.91 ± 0.10	0.76 ± 0.19
ICGV 98353 x KK 60-3	0.45 ± 0.31	0.36 ± 0.34	0.63 ± 0.25	0.76 ± 0.19	0.73 ± 0.20
Correlation (r)†	0.43**	0.40**	0.44**	0.33**	0.32**

* and ** significant at $P \leq 0.05$ and significant at $P \leq 0.01$, respectively.

† Correlations between well-watered conditions and drought conditions. ‡ Standard error.

Selection for HI, SCMR, and SLA would allow improvement of these traits and offers the potential to transfer desirable benefits such as increased WUE and drought tolerance to peanut. Evolutionary response to selection requires significant additive genetic variance for a given trait (Falconer and Mackay, 1996). Additive gene action has been the main factor responsible for variation in many agronomic traits in peanut. Previous studies reported that HI and SLA are mainly under additive genetic control and SCMR was found to be under the influence of both additive and non additive gene effects (Dwivedi et al., 1998; Jayalakshmi et al., 1999; Lal et al., 2006; Nigam et al., 2001; Suriharn et al., 2005). Hence, selection should be effective. Nigam et al. (2001) found that the selection for SLA and HI can be effective in early generations. They also suggest that the selection can be done in late generation to exploit the effect of additive x additive interaction.

Considerable genetic variation and high heritability estimates of physiological traits in this study indicate that selection for increasing drought resistance in peanut using HI, SCMR, and SLA should be successful. Although all physiological traits studied here were found to be highly heritable, genetic correlations between physiological trait and economic traits are needed in order to predict the response of yield and other agronomic traits from selection based on the physiological traits.

Phenotypic and genotypic correlations between drought resistance traits and agronomic traits

Significant correlations between drought resistance and agronomic traits were observed (Table 4). Genotypic (r_G) and phenotypic (r_P) correlations were similar, hence, only r_G is reported. Positive correlations were found between HI and pod yield, number of mature pods per plant, and seeds per pod (r_G = 0.48** to 0.78**). Positive correlations between SCMR and pod yield, biomass, and seed size were also significant (r_G = 0.23** to 0.34**). Results of this study indicate that selection for higher HI and SCMR will result in higher pod yield in peanut. SLA was negatively correlated with agronomic traits (r_G = -0.08* to -0.35**, respectively). Negative correlations between SLA and pod yield and biomass under stressed conditions were found (r_G = -0.14** to -0.35**), but were not observed under well-watered conditions. Small correlations between SLA and the yield components number of mature pods per plant and seed size were also found (r_G = -0.22* to 0.08*, respectively). Thus, genotypes with low SLA tend to have high pod yield, biomass, and large number mature pods per plant and seed size. Associations between SLA and agronomic traits were stronger under terminal drought conditions, indicating that selection for SLA under drought would be more effective than selection under non-stressed conditions.

Table 4 Genotypic (r_G) correlations between drought tolerance traits [harvest index (HI), SPAD chlorophyll meter reading (SCMR), and specific leaf area (SLA)] and agronomic traits [pod yield, biomass, number of pods/plant (PPP), seed/pod, and seed size] for 140 progeny lines of peanut under well-watered conditions and drought conditions in the dry season of 2006/07 and 2007/08.

Drought tolerance	Agronomic traits				
traits	Pod yield	Biomass	PPP	Seed/pod	Seed size
Well-watered conditions					
HI	0.66**	-0.34**	0.69**	0.52**	-0.20**
SCMR	0.34**	0.23**	0.00	-0.27**	0.31**
SLA	-0.05	0.01	0.08*	-0.01	-0.17**
Drought conditions					
HI	0.71**	-0.08*	0.78**	0.48**	0.05
SCMR	0.30**	0.28**	-0.08*	-0.11*	0.28**
SLA	-0.35**	-0.14**	-0.08*	-0.01	-0.22**

* and ** significant at $P \leq 0.05$ and significant at $P \leq 0.01$, respectively.

Genotypic associations in our study demonstrated that lower SLA and higher HI and SCMR were associated with increased pod yield. Hence, a breeding approach using these traits could be used to increase pod yield in peanut. Genotypic correlations between SCMR and SLA and agronomic traits were weak and found to be lower than r_G between HI and agronomic traits. However, SCMR and SLA are markedly less costly to evaluate and have been used to identify drought resistance in peanut. SLA was found to be associated with photosynthetic capacity. Low SLA expressed as thicker leaves usually has a higher density of chlorophyll per unit leaf area and hence a greater photosynthetic capacity than thinner leaves (Nageswara Rao et al., 1995; Nageswara Rao and Wright, 1994; Wright et al., 1994). Although SLA is affected by environment and genotype, the relationship between SLA and Δ and WUE is apparently stable across environments in peanut (Nageswara Rao and Wright, 1994; Wright et al., 1994; Upadhyaya, 2005). Furthermore, SLA was also found to be closely associated with HI (Songsri et al., 2008) and SCMR, a rapid assessment for leaf nitrogen and chlorophyll content in peanut (Nageswara Rao et al., 2001; Songsri et al., 2008; Upadhyaya, 2005). Significant correlations between SCMR and Δ, TE, and SLA have been observed over a wide range of environments (Arunyanark et al., 2008; Nigam and Aruna, 2008; Sheshshayee et al., 2006). Nigam et al. (2005) suggest that the SPAD chlorophyll meter, a portable hand held instrument, provides an easy opportunity to integrate a surrogate measure of WUE with pod yield in a drought resistance breeding program.

Because of low r_G between SCMR and SLA and agronomic traits, the use of a combination of physiological traits as a selection index may be advantageous to increase the effectiveness of drought resistance breeding programs. In addition, Bandyopadhyay et al. (1985) and Subbarao et al. (1995) suggested that breeding for drought resistance using integrated of a selection index based on physiological traits such as leaf area, specific leaf weight and leaf dry weight and components of yield was more efficient than an index based on yield components alone, and are more useful in crop improvement programs than single traits.

Genotypic correlations among drought resistance traits in well-watered and stressed conditions

Correlations between traits of interest can be used to determine if selection for one trait will have an effect on another trait. Genotypic associations among drought tolerance traits of 140 progeny lines under non-stressed and terminal drought conditions were calculated in this study (Table 5). Genotypic correlations among drought resistance traits were found under both water regimes. Positive and significant correlation between HI and SCMR were found under non-stressed (r_G = 0.15**) and stressed (r_G = 0.16**) conditions. The SLA was found to be inversely associated with SCMR and HI. Under terminal drought, SLA was negatively correlated with HI (r_G =

-0.33**) and SCMR (r_G = -0.31**). Under non-stressed conditions, negative correlation between SLA and SCMR was also observed (r_G = -0.42**). This confirms the earlier finding report by Songsri et al. (2008) and indicates that all three physiological traits can be used as indirect selection tools for each other, especially under stressed conditions.

Table 5 Genotypic (r_G) correlation among drought tolerance traits for progeny from all 4 peanut crosses (140 progeny lines) under field capacity (FC) and 1/3 available water (1/3AW) in the dry season of 2006/07 and 2007/08 (degrees of freedom = 556).

	1/3AW		FC	
	SCMR	SLA	SCMR	SLA
HI	0.15**	-0.33**	0.14**	-0.05
SCMR		-0.31**		-0.42**

* and ** significant at $P \leq 0.05$ and significant at $P \leq 0.01$, respectively.

Conclusions

Breeding for drought resistance in peanut requires the information of heritability and genetic associations among traits to be used in determining a proper selection scheme. Our results implies that HI, SLA, and SCMR are potentially useful as indirect selection index for terminal drought resistance because of their low G x E interactions, high heritabilities and significant correlations with pod yield and the other agronomic traits. Plant breeding approaches using these traits might be effective for improving terminal drought tolerance in peanut. This study found that selection for HI is expected to have a greater effect on yield and other agronomic traits than selection for SCMR and SLA. However, SCMR and SLA are easier to measure and should be more applicable in breeding programs with large segregating populations. To increase the effectiveness of breeding program for drought resistance, SCMR and SLA could be used as the first screening tools to reduce breeding material and then HI could be employed on the most promising material. In addition, the use of an integrated selection index based on these physiological traits might be profitable in breeding programmes.

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 Thailand Research Fund, and also support in part by the Basic Research for Supporting Groundnut Varietal Improvement for Drought Tolerance Project under the Thailand Research Fund. We thank the work of many people in field data collection and processing.

References

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

Bandyopadhyay, A., V. Arunachalan, and K. Venkajah. 1985. Efficient selection intensity in early generation index selection in groundnut (*Arachis hypogaea* L.). Theoretical and Applied Genetics 71: 300 - 304.

Blum, A. 1988. Plant breeding for stressed environments. CRC Press, Boca Raton, Florida.

Boote, K. J. 1982. Growth stage of peanut (*Arachis hypogaea* L.). Peanut Science 9: 35 - 40.

Bricke, A.A. 1989. MSTAT-C user's guide. Michigan State University, East Lansing, Michigan.

Cole, R. J., V. S. Sobolev, and J. W. Dorner. 1993. Potentially important sources of resistance to prevention of preharvest aflatoxin contamination in peanuts. Vol. 25 pp. 78. *In* Proceedings in American Peanut Research and Education Society, Huntsville, Alabama, USA.

Craufurd, P. Q., T. R. Wheeler, R. H. Ellis, R. J. Summerfield, and J. H. Williams. 1999. Effect of temperature and water deficit on water-use efficiency, carbon isotope discrimination, and specific leaf area in peanut. Crop Science 39: 136 - 142.

Cruickshank, A.L., A. Dowkiw, G.C. Wright, R.C. Nageswara Rao, and S.N. Nigam. 2004. Heritability of drought–resistance traits in peanut. *In* T. Fischer, N. Turner, J. Angus, L. McIntyre, M. Robertson, A. Borrell, and D. Lloyd (eds.), New directions for a diverse planet. Proceedings for the 4^{th} International Crop Science Congress. 26 September – 1 October 2004. Brisbane, Australia.

Doorenbos, J., and W.O. Pruitt. 1992. Calculation of crop water requirements, pp. 1–65. *In* FAO Irrigation and Drainage Paper No: 24. FAO of the United Nation. Rome, Italy.

Dorner, J. W., R. J. Cole, T. H. Sanders, and P. D. Blankenship. 1989. Interrelationship of kernel water activity, soil-temperature, maturity, and phytoalexin production in preharvest aflatoxin contamination of drought-stressed peanuts. Mycopathologia 105: 117 - 128.

Duncan, W. G., D. E. McCloud, R. L. McGraw, and K. J. Boote. 1978. Physiological aspects of peanut yield improvement. Crop Science 18: 1015 - 1020.

Dwivedi, S. L., S. N. Nigam, S. Chandra, and V. M. Ramraj. 1998. Combining ability of biomass and harvest index under short and long-day conditions. Groundnut. Annals of Applied Biology 133: 237 - 244.

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. Longman, London.

Girdthai, T., S. Jogloy, N. Vorasoot, C. Akkasaeng, S. Wongkaew, C. C. Holbrook, and A. Patanothai. 2010. Associations between physiological traits for drought tolerance and aflatoxin contamination in peanut genotypes under terminal drought. Plant Breeding (In press)

Gomez, K.A., and A.A. Gomez. 1984. Statistical procedures for agricultural research. 2nd ed. John Wiley & Sons: New York.

Hebbar, K. B., V. R. Sashidhar, M. Udhayakumar, R. Devendra, and R. C. Nageswara Rao. 1994. A comparative assessment of water use efficiency in groundnut (*Arachis hypogaea*) grown in containers and in the field under water-limited conditions. Journal of Agricultural Science 122: 429 - 434.

Holbrook, C. C., B. Z. Guo, D. M. Wilson, and P. Timper. 2009. The U.S. breeding program to develop peanut with drought tolerance and reduced aflatoxin contamination. Peanut Science 36: 50-53.

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

Holbrook, C. C., P. Ozias-Akins, P. Timper, D. M. Wilson, E. Cantonwine, B. Z. Guo, D. G. Sullivan, and W. Dong. 2008. Research from the coastal plain experiment station, Tifton, Georgia to minimize aflatoxin contamination in peanut. Toxin Reviews 27: 391 - 410.

Holland, J. B. 2001. Epistasis and plant breeding. Plant Breeding Reviews 21: 27 - 92.

Holland, J. B., W. E. Nyquist, and C. T. Cervartes-Martinez. 2003. Estimating and interpreting heritability for plant breeding: an update. Plant Breeding Reviews 22: 2 - 112.

Hubick, K. T., G. D. Farquhar, and R. Shorter. 1986. Correlation between water-use efficiency and carbon isotope discrimination in diverse peanut (*Arachis*) germplasm. Australian Journal of Plant Physiology 13: 803 - 816.

Hubick, K. T., R. Shorter, and G. D. Farquhar. 1988. Heritability and genotypic x environment interactions of carbon isotope discrimination and transpiration efficiency in peanut (*Arachis hypogaea* L.). Australian Journal of Plant Physiology 15: 799 - 813.

Jayalakshmi, V., C. Rajareddy, P. V. Reddy, and R. C. Nageswara Rao. 1999. Genetic analysis of carbon isotope discrimination and specific leaf area in groundnut (*Arachis hypogaea* L.). Journal of Oilseeds Research 16: 1 - 5.

Lal, C., K. Hariprasanna, A. L. Rathnakumar, H. K. Gor, and B. M. Chikani. 2006. Gene action for surrogate traits of water-use efficiency and harvest index in peanut (*Arachis hypogaea*). Annals of Applied Biology 148: 165 - 172.

Nageswara Rao, R. C., and G. C. Wright. 1994. Stability of the relationship between specific leaf area and carbon isotope discrimination across environments in peanut. Crop Science 34: 98 - 103.

Nageswara Rao, R. C., H. S. Talwar, and G. C. Wright. 2001. Rapid assessment of specific leaf area and leaf nitrogen in peanut (*Arachis hypogaea* L.) using a chlorophyll meter. Journal of Agronomy and Crop Science 186: 175 - 182.

Nageswara Rao, R. C., M. Udaykumar, G. D. Farquhar, H. S. Talwar, and T. G. Prasad. 1995. Variation in carbon isotope discrimination and its relationship to specific leaf area and ribulose–1, 5–bisphosphate carboxylase content in groundnut genotypes. Australian Journal of Plant Physiology 22: 545 - 551.

Nageswara Rao, R. C., Sardar Singh, M. V. K. Sivakumar, K. L. Srivastava, and J. H. Williams. 1985. Effect of water deficit at different growth phases of peanut. I. Yield responses. Agronomy Journal 77: 782 - 786.

Ndunguru, B. J., B. R. Ntare, J. H. Williams, and D. C. Greenberg. 1995. Assessment of groundnut cultivars for end-of-season drought tolerance in a Sahelian environment. Journal of Agricultural Science 125: 79-85.

Nigam, S. N., and R. Aruna. 2008. Stability of soil plant analytical development (SPAD) chlorophyll meter reading (SCMR) and specific leaf area (SLA) and their association across varying soil moisture stress conditions in groundnut (*Arachis hypogaea* L.). Euphytica 160: 111 - 117.

Nigam, S. N., H. D. Upadhyaya, S. Chandra, R. C. Nageswara Rao, G. C. Wright, and A. G. S. Reddy. 2001. Gene effects for specific leaf area and harvest index in three crosses of groundnut (*Arachis hypogaea*). Annals of Applied Biology 139: 301 - 306.

Nigam, S. N., S. Chandra, K. Rupa Sridevi, A. Manoha Bhukta, G. S. Reddy, R. C. Nageswara Rao, G. C. Wright, P. V. Reddy, M. P. Deshmukh, R. K. Mathur, M. S. Basu, S. Vasundhara, P. Vindhiya Varman, and A. K. Nagda. 2005. Efficiency of physiological trait-based and empirical selection approaches for drought tolerance in groundnut. Annals of Applied Biology 146: 433 - 439.

Ntare, B. R., and J. H. Williams. 1998. Heritability and genotype x environment interaction for yield and components of a yield model in segregating population of groundnut under semi-arid conditions. African Crop Science Journal 6(2): 119 - 127.

Passioura, J. B. 1986. Resistance to drought and salinity: Avenues for improvement. Australian Journal of Plant Physiology 13: 191 - 201.

Ravindra, V., P. C. Nautiyal, and Y. C. Joshi. 1990. Physiological analysis of drought resistance and yield in groundnut (*Arachis hypogaea* L.). Tropical Agriculture (Trinidad) 67: 290 - 296.

Sheshshayee, M. S., H. Bindumadhava, N. R. Rachaputi, T. G. Prasad, M. Udayakumar, G. C. Wright, and S. N. Nigam. 2006. Leaf chlorophyll concentration relates to transpiration efficiency in peanut. Annals of Applied Biology 148: 7 – 15.

Singh, M., S. Ceccarelli, and J. Hamblin. 1993. Estimation of heritability from varietal trials data. Theoretical and Applied Genetics 86: 437 - 441.

Singh, S., and M.B. Russel. 1981. Water use by maize/pigeonpea intercrop on a deep Vertisol, Vol. 1, pp. 271–282. *In* Proceedings of International workshop on pigeonpeas. 15–19 December 1980. ICRISAT Center. Patancheru, Andhra Pradesh, India.

Songsri, P., S. Jogloy, T. Kesmala, N. Vorasoot, C. Akkasaeng, A. Patanothai, and C. C. Holbrook. 2008. Heritability of drought resistance traits and correlation of drought resistance and agronomic traits in peanut. Crop Science 48: 2245 – 2253.

Subbarao, G. V., C. Johansen, A. E. Slinkard, R. C. Nageswara Rao, N. P. Saxena, and Y. S. Chauhan. 1995. Strategies for improving drought resistance in grain legumes. Critical Reviews in Plant Sciences 14: 469 - 523.

Surihan, B., A. Patanothai, and S. Jogloy. 2005. Gene effect for specific leaf area and harvest index in peanut (*Arachis hypogaea* L.). Asian Journal of Plant Sciences 4(6): 667 - 672.

Upadhyaya, H. D. 2005. Variability for drought resistance related traits in the mini core collection of peanut. Crop Science 45: 1432 - 1440.

Wallace, D. H ., J. P. Baudoin, J. Beaver, D. P. Coyne, D. E. Halseth, P. N. Masaya, H. M. Munger, J. R. Myers, M. Silberrnagel, K. S. Yourstone, and R. W. Zobel. 1993. Improving efficiency of breeding for higher crop yield. Theoretical and Applied Genetics 86: 27 - 40.

Wright, G.C., and R.C. Nageswara Rao. 1994. Groundnut water relations, pp. 281–325. *In* J. Smartt (ed.), The Groundnut Crop. A Scientific Basis for Improvement. Chapman and Hall: London.

Wright, G. C., K. T. Hubick, and G. D. Farquhar. 1988. Discrimination in carbon isotopes of leaves correlates with water-use efficiency of field-grown peanut cultivars. Australian Journal of Plant Physiology 15: 815 - 825.

Wright, G. C., K. T. Hubick, and G. D. Farquhar. 1991. Physiological analysis of peanut cultivar response to timing and duration of drought stress. Australian Journal of Agricultural Research 42: 453 - 470.

Wright, G. C., R. C. Nageswara Rao, and G. D. Farquhar. 1994. Water use efficiency and carbon isotope discrimination in peanut under water deficit conditions. Crop Science 34: 92 - 97.

CHAPTER VI

HERITABILITY OF, AND GENOTYPIC CORRELATIONS BETWEEN, AFLATOXIN TRAITS AND PHYSIOLOGICAL TRAITS FOR DROUGHT TOLERANCE UNDER END OF SEASON DROUGHT IN PEANUT (*Arachis hypogaea* L.)

Introduction

Preharvest aflatoxin contamination (PAC), induced by terminal drought and heat stress, in peanut (*Arachis hypogaea* L.) is an important quality problem with serious health concern worldwide. Aflatoxins, which are toxic secondary metabolites, are well recognized as potent carcinogenic, teratogenic and immunosuppressive substances (Turner et al., 2000; Wild and Hall, 2000; Hall and Wild, 2003) produced when toxigenic strains of the fungi *Aspergillus flavus* Link. ex Fries and *A. parasiticus* Speare grows on peanuts subjected to drought (Blankenship et al., 1984). Hence, a solution for eliminating or reducing PAC is necessary. Late season irrigation to alleviate drought stress of plants is effective in reducing PAC in the field (Dorner et al., 1989). However, cultivars with resistance to PAC are still needed, especially at locations where irrigation is not available.

Reduction of PAC through genetic manipulation has been attempted in breeding programs in many countries. However, identification of aflatoxin resistance traits and incorporation of pertinent traits into peanut has been a challenge for breeders. Genotype by environment (G x E) interactions are the main factor hindering the progress of breeding programs for lower PAC, and consistency and accuracy in field experimentation has been difficult to achieve (Anderson et al., 1995; Anderson et al., 1996; Holbrook et al., 1994).

This paper was submitted for publication in Field Crops Research.

Seed colonization can only be used as an initial screen because of the generally poor correlation between fungal growth and aflatoxin production. On the other hand, screening for resistance to PAC is also limited by the expense of directly measuring aflatoxin content. Thus, an indirect measure of PAC resistance in peanut is needed to accelerate progress in breeding programs.

PAC may be reduced with improved resistance to drought (Cole et al., 1993; Holbrook et al., 2008; 2009). Recent studies have shown a relationship of increased drought tolerance and reduced aflatoxin production (Arunyanark et al., 2009a; Girdthai et al., 2009; Holbrook et al., 2000). However, improvement of drought resistance based on yield is also hindered by high G x E interactions (Jackson et al., 1996; Araus et al., 2002). Drought resistance traits with lower G x E interactions are promising as indirect selection tools for improving resistance to PAC. Nigam and Aruna (2008) suggest that the SPAD chlorophyll meter reading (SCMR) and specific leaf area (SLA), are simple and stable drought resistance traits that are easy to measure in large breeding populations. Arunyanark et al. (2009a) found significant relationships between physiological traits for drought resistance such as SLA, root length density (RLD), and chlorophyll density (ChlD), with aflatoxin contamination under long term drought. Girdthai et al. (2009) also found that SLA, relative water content, ChlD, and drought stress ratings are the best traits to use as indirect selection tools for lower PAC under terminal drought conditions. Thus, physiological traits for drought tolerance may help breeders to reduce aflatoxin contamination in peanut.

Few studies to date have investigated the inheritance of aflatoxin traits in peanut. Arunyanark et al. (2009b) found moderate heritabilities for seed infection and aflatoxin contamination. They also found that aflatoxin traits were genetically correlated with drought tolerance traits, especially with HI, SLA and SCMR. However, they did not focus on terminal drought which is the most important period for PAC. The effectiveness of mechanisms of drought resistance is dependent on the timing and duration of drought stress. Drought escape mechanisms play an importance role under terminal drought which differs from long period drought (Subbarao et al., 1995; Clavel et al., 2004). From this perspective, the inheritance of aflatoxin traits under long term and terminal drought might be different.

To develop proper breeding strategies for incorporating resistance to drought and PAC, a breeder must identify sources of resistance, and determine the genetic control of resistance. Specific research on sources of resistance to aflatoxin in peanut has been conducted, but research on inheritance to elucidate the gene action controlling resistance to drought and PAC and to develop improved screening strategies has been limited. Hence, the objectives of the present study were to estimate the heritability of aflatoxin traits and genotypic and phenotypic correlations between drought resistance traits and PAC in peanut in order to predict indirect responses of PAC thought selection for drought resistance traits.

Materials and methods

Genetics materials and experimental design

Four populations developed by crossing 2 drought resistant genotypes, ICGV 98348 and ICGV 98353, with 2 commercial cultivars, KK 60-3 and Tainan 9, were used to study inheritance of resistance to drought and PAC. Two peanut genotypes [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) having low PAC with high pod yield (Girdthai et al., 2009). KK60-3 [late maturing (120 days to maturity) and large seeded type] selected for high PAC and biomass and Tainan 9 [early maturing (100 days to maturity) and medium seeded type] selected for high PAC and low biomass (Girdthai et al., 2009) are released cultivars and widely grown in Thailand. Four F_1 hybrids (ICGV 98348 x KK60-3, ICGV 98348 x Tainan 9, ICGV 98353 x KK60-3, and ICGV 98353 x Tainan 9) were obtained from the hybridization. The F_1 seeds were planted and their seeds harvested in bulk for each cross. In F_2 and F_3 generations, one pod was kept from each plant and bulked for each cross. Line separation was carried out in the F_4 generation. A total of 140 lines (35 lines for each cross) were randomly selected and multiplied in the F_5 generation.

Parental lines and the 140 lines from 4 crosses were evaluated in the $F_{4:6}$ and $F_{4:7}$ generations (F_4–derived lines in the F_6 and F_7 generations, respectively) under two soil moisture levels [field capacity (FC) and 1/3 available soil water (1/3 AW) at 80 days after planting (DAP) to final harvest] for two years in the dry season 2006/07 and repeated in the dry season 2007/08. A split plot design with four replications was used for both years at the Field Crop Research Station, Faculty of Agriculture Khon Kaen University located in Khon Kaen Province, Thailand (latitude 16° 28′ N, longitude 102° 48′ E, 200 m above sea level). Soil type is Yasothon Series (loamy sand, Ocix Paleustults) with 10.2 % soil moisture at FC and 3.1 % at permanent wilting point. Two soil moisture levels, FC (10.2 %) and 1/3 AW (5.5 %) in 0-60 cm depth were assigned as main plots, and peanut lines were laid out in subplots. Each entry was planted in five row plots with 3 m length. Spacing was 40 cm between rows and 20 cm between plants within the row.

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 large seeded genotypes were treated with ethrel (2-chloroethylphospphonic acid) 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 14 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 weeded 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 40 cm between drip lines at 10 cm below the soil surface midway between peanut rows to supply water to the crop. Drip lines were fitted with a pressure valve and a water meter to ensure a uniform supply of the required amounts of water. Soil water level was maintained at FC at 0-60 cm depth. This soil depth should reasonably cover the majority of the rooting zone. In 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 each plot. 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.

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 pod zone. The aflatoxin producing strain of *A. flavus* used in this study was kindly provided by the laboratory of Suranaree University of Technology, Nakhonratchasima province, Thailand. 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 °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.

Data collection

Soil moisture status and weather data

Soil moisture in each main 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, and 55-60 cm. Readings were taken at two positions in each main plot. The measurement before planting was used for calculating the correct amount of water to be applied for the crop. Soil moisture volume fraction was also monitored at 10 day intervals from planting to final harvest using a neutron moisture meter (Type I.H. II SER, no. N0152, Ambe Didcot Instruments Co. Ltd, Abingdon, UK). Five aluminium access tubes were installed in each main plot. Readings were taken in access tubes from the depth of 30-90 cm at 30 cm intervals.

Weather data during two cropping seasons were recorded daily from sowing until final harvest by a meteorological station located 600 m away from the experimental field. Forty mm of the total amount of rainfall was recorded during 80-100 DAP in 2006/07, and 22.7 mm was recorded during the same period in 2007/08 (Figure 1). Air temperature, relative humidity and evaporation in 2006/07 were higher than in the 2007/08, especially during the water stress period. During stress period (80 DAP to final harvest), mean evaporation was 6.0 and 5.0 mm in 2006/07 and 2007/08, respectively. The maximum and minimum air temperature ranged from 11.8 to 38.5 ^{o}C in 2006/07 and 14.5 to 35.2 ^{o}C in 2007/08, being lower during 80–110 DAP in 2007/08. Relative humidity ranged from 54 to 93 % in 2006/07 and from 57 to 92 % in 2007/08. The seasonal mean solar radiation was 0.13 and 0.11 Cal cm^{-2} in 2006/07 and 2007/08, respectively.

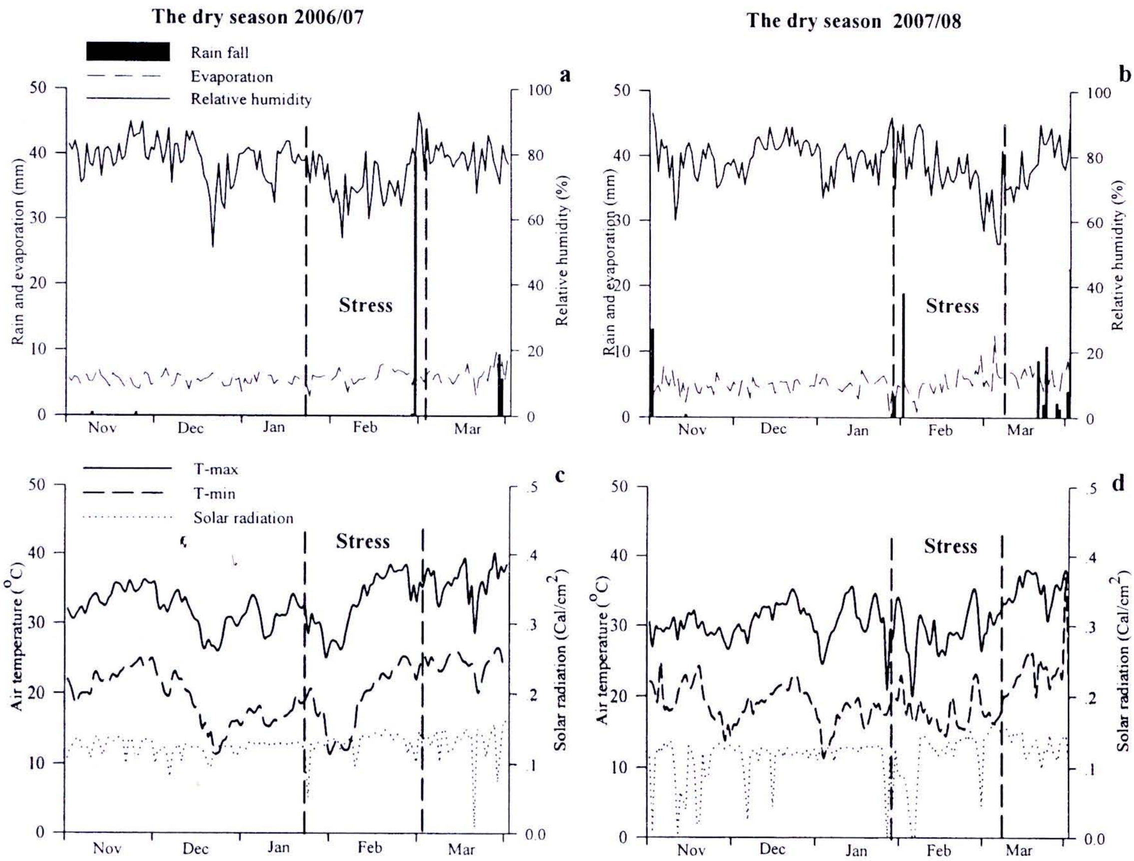

Figure 1 Relative humidity (%) (a and b), pan evaporation (mm) (a and b), rainfall (mm) (a and b), maximum and minimum air temperature (^{o}C) (c and d), and solar radiation (Cal/cm^2) (c and d) during the crop growth period in 2006/07 (a and c) and in 2007/08 (c and d).

SPAD chlorophyll meter reading and specific leaf area

Data were recorded for SCMR and SLA at 80, 90, and 100 DAP. Five plants were randomly selected in each plot to record SCMR and SLA following the procedure described by Nageswara Rao et al. (2001). The second fully expanded leaves were detached from the chosen plants at 10-12 AM and brought to the laboratory in zipped polythene bags for recording observations. SCMR was recorded using a Minolta SPAD-502 meter (Minolta SPAD-meter, Tokyo, Japan) on the four leaflets from each leaf. An average SCMR for each plot was derived from 20 single observations (four leaflets x 5 plants plot^{-1}). In recording the SCMR, care was taken to ensure that the SPAD meter sensor fully covered the leaf lamina and that interference from veins and midribs was avoided.

After recording SCMR, the leaf area of all five sampled plants was measured with a leaf area meter (LI 3100C Area Meter, LI-COR Inc., USA) after which the leaves were dried in an oven at 80°C for at least 48 hours to determine leaf dry weight. Immediately after drying, the leaves were weighed and the SLA was derived as leaf area per unit leaf dry weight (cm^2 g^{-1}).

Biomass and pod yield

For each plot excluding boarder plants, three rows with 2.6 m in length (3.12 m^2) were harvested at maturity (R8) (Boote, 1982), and their pods and roots were removed before taking fresh shoot weight in the field. Five plants were randomly selected for measuring shoot fresh weight and then oven dried at 80°C for at least 48 hours and dry weight was measured. Shoot dry matter was then calculated and used in determining shoot dry weight for a plot. Pod yields were weighed after air drying to approximately 7-8 % moisture content. HI was computed by the following formula:

HI = pod weight / total biomass

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**

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 modified from that used by Chu et al. (1987) and Chu (1989). After grinding, a 20 g subsample was placed in 100 ml of methanol-dimethyl formamide – water solution (70:1:29 % v/v). The sample was then homogenized at high speed in an electric grinder for 3 min, and allowed to settle for

10 minutes. Microtitre plate (Microtitre plate. – "NUNC" maxisop®, 96 wells) with antigens on the surface (solid phase) was used for this assay. The wells of a microtitre plate were coated with aflatoxin B_1 – oxime – BSA (bovine serum albumin) (Sigma A-6655) and incubated in the dark at room temperature (25 °C) for 60 minutes and then washed 3 times. The supernatant from each sample was collected and then loaded simultaneously with a competitive agent (anti – aflatoxin B_1 – BSA – HRP (Horse radish peroxidase) conjugate (Sigma A-2681)) into wells of the microtitre plate. After incubation and washing, the amount of enzyme on binding site of the anti – aflatoxin B_1 – BSA – HRP which bound in the surface of each well was determined by incubation with a specific substrate solution. The optical density was read at a wavelength of 492 nm by an ELISA reader. Standard sample as 1000, 500, 250, 125, 62.5, 31.2, 15.6, 7.8, and 0 ppb with 4 replicates were also analyzed simultaneously with the samples in each microtitre plate for construction of a standard absorbance concentration curve on a semi log graph. The relative amount of aflatoxin B_1 of the sample was then calibrated by comparing with those of the standard curve.

Statistical analysis

Analysis of variance was performed for each trait in each year following a split plot design (Gomez and Gomez, 1984). Because water regime x genotype interaction was significant, each water regime was analyzed separately according to a randomized complete block design (RCBD) (Gomez and Gomez, 1984), and data under drought treatment, excluding the data from well-irrigated treatment, were reported herein. Kernel infection and aflatoxin contamination were also analyzed only under drought conditions. Calculation procedures were conducted using Statistix 8 (Analytical Software, Tallahassee, FL, USA).

As the evaluation of heritability estimates was conducted in late generations (F_6 and F_7) of segregating materials when most genes were nearly fixed in individual genotypes, it would be expected that additive genetic variances for the traits under study were fixed through generation advance (Holland, 2001). Estimates of broad-sense heritability for the four crosses were calculated by partitioning variance components of family mean squares to pooled environment variance (δ^2_E) and genotypic variance (δ^2_G), and then broad-sense heritability estimates (h^2_b) were calculated as follows (Holland et al., 2003):

$$h^2_b = \delta^2_G / \delta^2_P$$

$$\delta^2_P = \delta^2_G + \delta^2_{GE} /e + \delta^2_E /re,$$

where h^2_b = broad sense heritability, δ^2_G = genotypic variation, δ^2_P = phenotypic variation, r = no. of replications, and e = no. of environments. The standard error (SE) of heritability (Singh et al., 1993) for drought tolerance traits and PAC were calculated to give a measure of the precision of the estimate.

Phenotypic and genotypic correlations between aflatoxin traits and drought tolerance traits were calculated based on progeny means (140 lines) following the methods of Falconer and Mackay (1996), more descriptive information could also be seen in Songsri et al. (2008).

Results

Soil moisture status

Soil moisture data measured by Neutron probe and Gravimetric method were similar and showed significant difference between water treatments for both years (Figure 2). Average soil moisture under the drought conditions slightly decreased from 60 DAP to 80 DAP. At 80 DAP, soil moisture under drought treatment (5.7 % in both years) were lower than the irrigated conditions (11.5 % in 2006/07 and 10.2 % in 2007/08, respectively). The soil moisture content of both treatments was held fairly constant from 80 DAP until harvest.

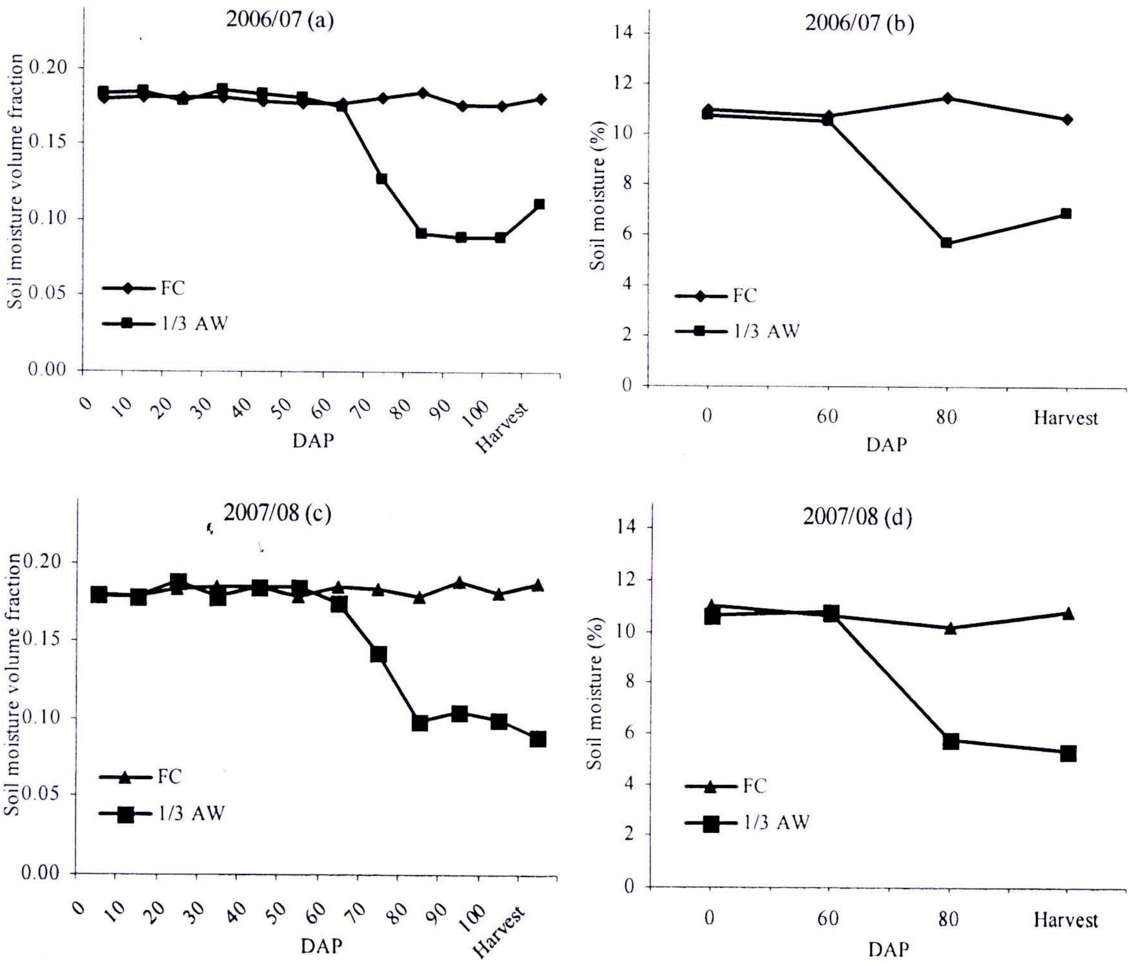

Figure 2 Soil moisture volume fraction (a and c) at planting, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 days after planting (DAP), and at final harvest and gravimetric soil moisture content (b and d) at planting, 60, 80 DAP and at final harvest under different water regimes [field capacity (FC) and 1/3 available water (1/3 AW)] average from 0-60 cm depth in 20062007 (a and b) and 2007/2008 (c and d).

Combined analysis of variance

Large and significant differences between the 140 genotypes for aflatoxin traits were found ($P \leq 0.01$) indicating genetic variations for these characters (Table 1). This also reveals that the heritability of the traits can be estimated in these populations. Difference in years for seed infection and aflatoxin contamination were significant ($P \leq 0.05$ to $P \leq 0.01$). Interaction effects of year x genotypes (Y x G) for seed infection and aflatoxin contamination were also significant ($P \leq 0.05$ to

P ≤ 0.01). Y x G interaction effects for seed infection was higher than for aflatoxin contamination. The significant G x E interaction indicates that aflatoxin traits across environments are inconsistent among genotypes.

Table 1 Mean square from the combined ANOVA for aflatoxin traits [*A. flavus* infection and aflatoxin contamination] at final harvest under terminal drought of 140 genotypes in the dry season of 2006/07 and 2007/08.

Source of variation	df	*A. flavus* infection	Aflatoxin contamination
Year (Y)	1	324,212 **	7,711,945 *
Rep. within Y	6	1,256	827,147
Genotypes (G)	139	613 **	64,896 **
Y x G	139	416 **	44,309 *
Pooled error	834	412	17,184

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

Yield, physiological traits, and aflatoxin traits under terminal drought

Wide ranges for pod yield and biomass were observed and reported herein (Table 2). Differences among genotypes for pod yield and total biomass were greater in 2006/07 than in 2007/08 (as indicated by the wide ranges of means). Average pod yield in 2007/08 (2,180 kg ha^{-1}) was higher than in 2006/07 (2,002 kg ha^{-1}). In 2006/07, however, average total biomass was 7,354 kg ha^{-1}, and higher than in 2007/08 (7,210 kg ha^{-1}). Mean and range of SCMR were not different between years. Wide ranges of SLA and aflatoxin traits in 2007/08 were found. In 2007/08, means of SLA and aflatoxin traits were higher than in 2006/07 with the exception of SLA at 100 DAP.

Table 2 Ranges and means of pod yield, total biomass, physiological traits [specific leaf area (SLA) and SPAD chlorophyll meter reading (SCMR) at 80, 90, and 100 days after planting(DAP)], and aflatoxin traits under terminal drought conditions of four peanut crosses in the dry season of 2006/07 and 2007/08.

Traits	In 2006/07			In 2007/08		
	Range	Mean	SE	Range	Mean	SE
Pod yield (kg h^{-1})	252 - 4696	2002	37	552 - 4250	2180	31
Biomass (kg h^{-1})	1825 - 15426	7354	102	3302 - 11420	7210	73
SCMR 80 DAP	34 - 55	44	0.165	35 - 55	43	0.157
SCMR 90 DAP	32 - 55	45	0.155	38 - 55	47	0.143
SCMR 100 DAP	35 - 60	48	0.184	39 - 60	48	0.157
SLA 80 DAP (cm^2 g^{-1})	105 - 173	135	0.606	126 - 236	179	0.980
SLA 90 DAP (cm^2 g^{-1})	101 - 160	124	0.494	113 - 198	151	0.687
SLA 100 DAP (cm^2 g^{-1})	113 - 181	144	0.553	99 - 162	124	0.521
A. flavus infection (%)	21 - 42	37	3.1	35 - 56	44	3.5
Aflatoxin contamination (ppb)	180 - 1120	653.3	7.96	258 - 1538	819.3	11.26

SE, Standard error for genotypes means.

Heritability of aflatoxin traits

Heritabilities for seed infection and aflatoxin contamination were low to moderate (Table 3). In this study, the heritability estimates for seed infection and aflatoxin contamination were not significantly different. The heritabilities for seed infection ranged from 0.48 to 0.58, and the heritabilities for aflatoxin contamination ranged from 0.24 to 0.68.

Table 3 Heritability estimates for aflatoxin traits [*A. flavus* infection and aflatoxin contamination] at final harvest under terminal drought conditions of four peanut crosses in the dry season of 2006/07 and 2007/08.

	Broad sense heritability	
Peanut crosses	*A. flavus* infection	Aflatoxin contamination
ICGV 98348 x Tainan 9	0.48 ± 0.30[†]	0.68 ± 0.23
ICGV 98348 x KK 60-3	0.58 ± 0.29	0.30 ± 0.33
ICGV 98353 x Tainan 9	0.51 ± 0.32	0.40 ± 0.30
ICGV 98353 x KK 60-3	0.56 ± 0.28	0.24 ± 0.34
4 cross	0.50 ± 0.28	0.32 ± 0.30

[†] Standard error.

Phenotypic and genotypic correlations between aflatoxin traits and drought tolerance traits

Significant correlations between aflatoxin traits and DTI (PY), DTI (BIO), HI, biomass and pod yield under terminal drought conditions were found (Table 4). Genotypic correlations (r_G) between traits were stronger than phenotypic correlations (r_P). Correlations of aflatoxin contamination with DTI (BIO) were highest (r_P = -0.23**, r_G = -0.57**), followed by correlations with HI (r_P = -0.20**, r_G = -0.36**) and DTI (PY) (r_P = -0.13**, r_G = -0.25**). Weak correlations between pod yield and aflatoxin contamination were found (r_P = -0.14**, r_G = -0.08*), but significant correlations were not found between pod yield and *A. flavus* infection. Positive associations between biomass and aflatoxin traits were also significant (r_P = 0.32**, r_G = 0.41** to 0.53**). Correlations between *A. flavus* infection and drought tolerance traits were weak (r_P = -0.07* to -0.25**, r_G = -0.11** to 0.41**), and lower than correlation between aflatoxin contamination and drought tolerance traits (r_P = -0.13** to -0.23**, r_G = -0.08* to 0.57**).

Table 4 Phenotypic (r_P) and genotypic (r_G) correlations between pod yield, biomass, and drought tolerance index for pod yield (DTI (PY)) and biomass (DTI (BIO)), and harvest index (HI) with aflatoxin traits [*A. flavus* infection and aflatoxin contamination] from all progeny lines under drought in the dry season of 2006/07 and 2007/08.

	A. flavus infection				Aflatoxin contamination			
Traits	r_P		r_G		r_P		r_G	
Pod yield	0.02		-0.06		-0.14	**	-0.08	*
Biomass	0.32	**	0.41	**	0.06		0.53	**
DTI (BIO)	-0.07	*	-0.11	**	-0.23	**	-0.57	**
DTI (PY)	-0.15	**	-0.19	**	-0.13	**	-0.25	**
HI	-0.25	**	-0.28	**	-0.20	**	-0.36	**

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

†DTI were calculated by the ratio of stressed

(1/3 available water (AW)) / non-stressed (field capacity (FC)) conditions.

Phenotypic and genotypic correlations between physiological traits and aflatoxin traits

Close associations between physiological traits for drought resistance and aflatoxin traits were found (Table 5). Phenotypic correlation between SLA and PAC (r_P = 0.40** to 0.46**) and genotypic correlations between SCMR and SLA and PAC (r_G = -0.45** to 0.81**) were moderate to high. Phenotypic and genotypic correlations between physiological traits and *A. flavus* infection (r_P = -0.10** to 0.29**, r_G = -0.11** to 0.45**) and phenotypic correlation between SLA and PAC (r_P = -0.30** to -0.40**) were rather low. Associations between physiological traits and aflatoxin contamination were higher than associations between physiological traits and *A. flavus* infection, indicating that selection for low SLA and high SCMR would have an effect on PAC more than on *A. flavus* infection. Positive correlations between SLA at 80, 90, and 100 DAP and *A. flavus* infection and PAC were significant (r_P = 0.13** to 0.46**, r_G = 0.26** to 0.81**). This indicated that selection for lower SLA or thicker leaf will result in lower PAC and seed infection in peanut. SCMR was negatively correlated with aflatoxin traits (r_P = -0.10** to -0.40**, r_G = -0.11** to 0.66**). Thus, genotypes with high SCMR or leaf nitrogen content tend to have low PAC. SLA at 100 DAP and SCMR at 80 DAP seem to be the best physiological traits for lower PAC because of high correlation with PAC.

Table 5 Phenotypic (r_P) and genotypic (r_G) correlations between drought tolerance traits [specific leaf area (SLA) and SPAD chlorophyll meter reading (SCMR)] at 80, 90, and 100 days after planting (DAP) and aflatoxin traits [*A. flavus* infection and aflatoxin contamination] at final harvest from all 4 peanut crosses (140 progeny lines) under drought conditions in the dry season of 2006/07 and 2007/08.

Drought tolerance traits	Aflatoxin traits			
	A. flavus infection		Aflatoxin contamination	
	r_P	r_G	r_P	r_G
SCMR				
80 DAP	-0.06	-0.23 **	-0.40 **	-0.66 **
90 DAP	-0.10 **	-0.31 **	-0.31 **	-0.45 **
100 DAP	0.03	-0.11 **	-0.30 **	-0.51 **
SLA				
80 DAP	0.04	0.26 **	0.40 **	0.68 **
90 DAP	0.13 **	0.37 **	0.41 **	0.57 **
100 DAP	0.29 **	0.45 **	0.46 **	0.81 **

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

Discussions

Aflatoxin production in peanut appeared to be greatly influenced by the environment. Due to environmental and G x E interaction effects, genotypes reported to have resistance to aflatoxin production have been shown not to have consistency across different growing environments (Anderson et al., 1995; Anderson et al., 1996; Holbrook et al., 1994). G x E interactions of aflatoxin traits found in this study confirmed that field-based selection approaches for eliminating PAC in peanut will be difficult to achieve. Moreover, heritabilities of *Aspergillus* infection and aflatoxin contamination in this study were rather low. Thus, the expected genetic gains from selection for aflatoxin traits will be low. Estimates of low to moderate heritabilities for aflatoxin traits were generally in agreement with those previously reported by Arunyanark et al. (2009b). Utomo et al. (1990) also reported that resistance to seed infection and aflatoxin production in peanut inform the crosses AR-4 x NC 7 and GFA-2 x NC 7 are controlled by difference genes with low heritabilities (ranged from 0.20 - 0.63). Mixon (1976), however, found high heritability estimates for seed infection in a population from the cross PI 337409 x PI 331326.

The ability to maintain pod and plant moisture contents under drought stress has been proposed as a main mechanism that can help to maintain the capacity of plants to produce stilbene phytoalexin preventing PAC (Dorner et al., 1989; Wotton and Strange, 1985). Hence, a possible means of reducing PAC in peanut is the use of cultivars with improved resistance to drought stress. Breeding progress using this approach might be accelerated if the physiological traits for drought resistance that contribute to, or are associated with, aflatoxin resistance could be identified.

Researchers have demonstrated the correlations between drought tolerance traits and aflatoxin contamination in order to identify an indirect selection tool for eliminating PAC in peanut (Arunyanark et al., 2009a; Girdthai et al., 2009; Holbrook et al., 2000). Holbrook et al. (2000) found the significant relationships between the drought resistance traits, canopy temperature and visual stress rating, with PAC, and proposed that these traits might be useful in indirectly selecting for lower PAC. However, Girdthai et al. (2009) suggested that although drought stress rating seems to be a fast and inexpensive tool, the correlation to PAC was not consistent. Therefore, drought stress ratings might be used in combination with other physiological traits as indirect selection tools for lower aflatoxin contamination. They found that associations between SLA and canopy temperature with PAC were more consistent and stronger. Arunyanark et al. (2009a) also found that PAC was associated well with SLA, SCMR, root length density, and drought tolerance indices under long period drought conditions. SLA and SCMR seem to be the best indirect selection criteria for reducing PAC because these traits have high heritability, and are less variable and less expensive to measure (Songsri et al., 2008). Information on the genetic correlations between drought tolerance traits and *Aspergillus* infection and aflatoxin contamination should be useful in determining the most effective breeding scheme for developing peanut cultivars with reduced aflatoxin contamination.

Genotypic associations between aflatoxin traits and drought tolerance traits found in this study demonstrated that genotypes with high DTI (PY), DTI (BIO), and HI tend to have low *A. flavus* infection and PAC. This implied that the ability to maintain higher biomass and pod yield during drought periods may be important traits enabling cultivars to resist aflatoxin production. Weak correlations between PAC and pod yield in this study confirmed the finding of Holbrook et al. (2000) who found a negative phenotypic correlation between aflatoxin contamination and yield under drought stressed conditions. Hence, selection of genotypes which have higher yield under drought conditions could also lower aflatoxin contamination compared to lower yielding genotypes.

Close associations between physiological traits for drought resistance and aflatoxin traits reported herein implied that SLA, and SCMR are potentially useful as indirect selection tools to reduce PAC. SLA and SCMR have been used to identify drought resistant genotypes in breeding programs (Nageswara Rao and Wright, 1994; Nigam and Aruna, 2008; Wright et al., 1994). Significant correlations between SCMR and SLA with other physiological traits for drought tolerance, such as carbon isotope discrimination, harvest index, and transpiration efficiency, have been observed over a wide range of environments (Arunyanark et al., 2008; Nigam and Aruna, 2008; Sheshshayee et al., 2006). SLA was associated with variation in photosynthetic capacity and chlorophyll density expressed as high SCMR (Wright and Nageswara Rao, 1994; Nageswara Rao et al., 1995; 2001). Therefore, peanut genotypes with low

SLA, or thicker leaves have more photosynthetic capacity or chlorophyll density. Through this study we have found that selection for high SCMR and low SLA is expected to have a greater effect on *A. flavus* infection and PAC than selection for the other drought resistance traits. Moreover, SCMR and SLA are less variable and easier to measure than aflatoxin and drought resistance traits based on yield. Hence, these traits should be more applicable in breeding programs with large segregating populations.

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 Thailand Research Fund, and also support in part by the Basic Research for Supporting Groundnut Varietal Improvement for Drought Tolerance Project under the Thailand Research Fund. We thank the work of many people in field data collection and processing.

References

Anderson, W.F., C.C. Holbrook, and D.M. Wilson. 1996. Development of greenhouse screening for resistance to *Aspergillus parasiticus* infection and preharvest aflatoxin contamination in peanut. Mycopathologia 135: 115–118.

Anderson, W.F., C.C., Holbrook, D.M. Wilson, and M. E. Matheron. 1995. Evaluation of preharvest aflatoxin contamination in several potentially resistant peanut genotypes. Peanut Science 22: 29–32.

Araus, J.L., G.A. Slafer, M.P. Reynolds, and C. Royo. 2002. Plant breeding and drought in C3 cereals: what should we breed for?. Annals of Botany 89: 925–940.

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

Arunyanark, A., S. Jogloy, S. Wongkaew, C. Akkasaeng, N. Vorasoot, G.C. Wright, Rao C.N. Rachaputi, and A. Patanothai. 2009a. Association between aflatoxin contamination and drought tolerance traits in peanut. Field Crops Research 114: 14-22.

Arunyanark, A., S. Jogloy, S. Wongkaew, C. Akkasaeng, N. Vorasoot, T. Kesmala, and A. Patanothai. 2009b. Heritability of aflatoxin resistance traits and correlation of aflatoxin resistance and drought tolerance traits under drought conditions in peanut. Field Crop Research (In press)

Blankenship, P.D., R.J. Cole, T.H. Sanders, and R.A. Hill. 1984. Effect of geocarposphere temperature on pre–harvest colonization of drought–stressed peanuts by *Aspergillus flavus* and subsequent aflatoxin contamination. Mycopathologia 85: 69–74.

Boote, K. J. 1982. Growth stage of peanut (*Arachis hypogaea* L.). Peanut Science 9: 35–40.

Chu, F.S. 1989. Current immunochemical methods for analysis of aflatoxin in groundnut and ground products; aflatoxin contamination of groundnut, pp. 161–172. *In* D. McDonald, V.K. Mehan, and S.D. Hall (eds.), Proceedings of the International Workshop in Aflatoxin Contamination of Groundnuts. 6–9 Oct. 1987. ICRISAT center, Patancheru, Andhra Pradesh, India.

Chu, F.S., T.S.L. Fan, G.S. Zhang, and Y.C. Xu. 1987. Improved enzyme-linked immunosorbent assay for aflatoxin B1 agricultural commodities. Journal of the Association of Official Analytical Chemists 70: 1125–1128.

Clavel, D., B. Sarr, E. Marone, and R. Ortiz. 2004. Potential agronomic and physiological traits of Spanish groundnut varieties (*Arachis hypogaea* L.) as selection criteria under end-of cycle drought conditions. Agronomie 24: 101-111.

Cole, R. J., V. S. Sobolev, and J. W. Dorner. 1993. Potentially important sources of resistance to prevention of preharvest aflatoxin contamination in peanuts. Vol. 25 pp. 78. *In* Proceedings in American Peanut Research and Education Society, Huntsville, Alabama, USA.

Doorenbos, J., and W.O. Pruitt. 1992. Calculation of crop water requirements, pp. 1–65. *In* FAO Irrigation and Drainage Paper No: 24. FAO of the United Nation. Rome, Italy.

Dorner, J.W., R.J. Cole, T.H. Sanders, and P.D. Blankenship. 1989. Interrelationship of kernel water activity, soil–temperature, maturity, and phytoalexin production in preharvest aflatoxin contamination of drought–stressed peanuts. Mycopathologia 105: 117–128.

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. Longman, London.

Girdthai, T., S. Jogloy, N. Vorasoot, C. Akkasaeng, S. Wongkaew, C. C. Holbrook, and A. Patanothai. 2010. Associations between physiological traits for drought tolerance and aflatoxin contamination in peanut genotypes under terminal drought. Plant Breeding doi:10.1111/j.1439-0523.2009.01738.x.

Gomez, K.A., and A.A. Gomez. 1984. Statistical procedures for agricultural research. 2nd ed. John Wiley & Sons: New York.

Hall, A.J., and C. P. Wild. 2003. Liver cancer in low and middle income countries: prevention should target vaccination, contaminated needles and aflatoxins. British Medical Journal 326: 994–995.

Holbrook, C. C., B. Z. Guo, D. M. Wilson, and P. Timper. 2009. The U.S. breeding program to develop peanut with drought tolerance and reduced aflatoxin contamination. Peanut Science 36: 50-53.

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

Holbrook, C.C., M. E. Matheron, D.W. Wilson, W.F. Anderson, M. E. Will, and A. J. Noden. 1994. Development of a large-scale field screening system for resistance to preharvest aflatoxin contamination. Peanut Science 21: 20-22.

Holbrook, C. C., P. Ozias-Akins, P. Timper, D. M. Wilson, E. Cantonwine, B. Z. Guo, D. G. Sullivan, and W. Dong. 2008. Research from the coastal plain experiment station, Tifton, Georgia to minimize aflatoxin contamination in peanut. Toxin Reviews 27: 391-410.

Holland, J. B. 2001. Epistasis and plant breeding. Plant Breeding Reviews 21: 27 - 92.

Holland, J. B., W. E. Nyquist, and C. T. Cervartes-Martinez. 2003. Estimating and interpreting heritability for plant breeding: an update. Plant Breeding Reviews 22: 2 - 112.

Jackson, P., M. Roberston, M. Cooper, and G. Hammer. 1996. The role of physiological understanding in plant breeding; from a breeding perspective. Field Crop Research 49: 11−39.

Mixon, A. C. 1976. Peanut breeding strategy to minimize aflatoxin contamination. Journal of American Peanut Research and Education Association 8: 54-58.

Nageswara Rao, R. C., and G. C. Wright. 1994. Stability of the relationship between specific leaf area and carbon isotope discrimination across environments in peanut. Crop Science 34: 98 - 103.

Nageswara Rao, R. C., H. S. Talwar, and G. C. Wright. 2001. Rapid assessment of specific leaf area and leaf nitrogen in peanut (*Arachis hypogaea* L.) using a chlorophyll meter. Journal of Agronomy and Crop Science 186: 175 - 182.

Nageswara Rao, R.C., M. Udaykumar, G.D. Farquhar, H.S. Talwar, and T.G. Prasad. 1995. Variation in carbon isotope discrimination and its relationship to specific leaf area and ribulose–1, 5–bisphosphate carboxylase content in groundnut genotypes. Australian Journal Plant Physiology 22: 545–551.

Nautiyal, P.C., R.C. Nageswara Rao, and Y.C. Joshi. 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 Crops Research 74: 67–79.

Nigam S. N., and R. Aruna. 2008. Stability of soil plant analytical development (SPAD) chlorophyll meter reading (SCMR) and specific leaf area (SLA) and their association across varying soil moisture stress conditions in groundnut (*Arachis hypogaea* L.). Euphytica 160: 111 - 117.

Sheshshayee, M. S., H. Bindumadhava, N. R. Rachaputi, T. G. Prasad, M. Udayakumar, G. C. Wright, and S. N. Nigam. 2006. Leaf chlorophyll concentration relates to transpiration efficiency in peanut. Annals of Applied Biology 148: 7 – 15.

Singh, M., S. Ceccarelli, and J. Hamblin. 1993. Estimation of heritability from varietal trials data. Theoretical and Applied Genetics 86: 437 - 441.

Singh, S., and M.B. Russel. 1981. Water use by maize/pigeonpea intercrop on a deep Vertisol, Vol. 1, pp. 271–282. *In* Proceedings of International workshop on pigeonpeas. 15–19 December 1980. ICRISAT Center. Patancheru, Andhra Pradesh, India.

Songsri, P., S. Jogloy, T. Kesmala, N. Vorasoot, C. Akkasaeng, A. Patanothai, and C. C. Holbrook. 2008. Heritability of drought resistance traits and correlation of drought resistance and agronomic traits in peanut. Crop Science 48: 2245 – 2253.

Subbarao, G. V., C. Johansen, A. E. Slinkard, R. C. Nageswara Rao, N. P. Saxena, and Y. S. Chauhan. 1995. Strategies for improving drought resistance in grain legumes. Critical Reviews in Plant Sciences 14: 469 - 523.

Turner, P.C., M. Mendy, H. Whittle, M. Fortuin, A.J. Hall, and C.P. Wild. 2000. Hepatitis B infection and aflatoxin biomarker levels in Gambian children. Tropical Medicine and International Health 5: 837–41.

Utomo, S.D., W.F. Anderson, J.C. Wynne, M.K. Beute, W.M. Jr. Hagler, and G.A. Payne. 1990. Estimates of heritability and correlation among three mechanisms of resistance to Aspergillus parasiticus in peanut. Vol. 22 pp. 26. *In* Proceedings of the American Peanut Research and Education Society. Stone Mountain, Georgia, USA.

Wild, C.P., and A.J. Hall. 2000. Primary prevention of hepatocellular carcinoma in developing countries. Mutation Research 462: 381–393.

Wotton, H. R., and R. N. Strange. 1985. Circumstantial evidence for phytoalexin involvement in the resistance of peanuts to *Aspergillus flavus*. Journal of General Microbiology 131: 487-494.

Wright, G.C., and R.C. Nageswara Rao. 1994. Groundnut water relations, pp. 281–325. *In* J. Smartt (ed.), The Groundnut Crop. A Scientific Basis for Improvement. Chapman and Hall: London.

Wright, G. C., R. C. Nageswara Rao, and G. D. Farquhar. 1994. Water use efficiency and carbon isotope discrimination in peanut under water deficit conditions. Crop Science 34: 92 - 97.

CHAPTER VII
GENERAL DISCUSSION AND CONCLUSION

Breeding for drought tolerance is an important strategy to increase long-term productivity, and reduce PAC in peanut under drought prone environments. However, improvement of drought resistance and lower PAC is particularly timely, given the huge developments in this area in the past decade. Utilizing drought-resistant peanut genotypes could overcome the PAC problem which is a serious health concern worldwide. In breeding programmes worldwide, selection for drought resistance has been primarily on the basis of yield and biomass. Progress from this procedure has been slow because of low heritability and high G x E interactions of the trait. Breeding approaches using physiological traits have been proposed to improve selection efficiency for superior drought-tolerant genotypes and found to be an indirect selection tools for lower PAC. The information on physiological traits contributing to yield and preventing the seed from fungus infection and PAC under terminal drought stress might reveal the underlying mechanisms from which improved strategies could be developed to enhance the effectiveness and progress of breeding programmes for drought resistance in peanut. Hence, indirect selection tools for drought resistance and lower PAC need to be developed and tested.

This thesis research was comprised of four series of experiments including screening techniques for root characteristics, identifying physiological traits for drought resistance as indirect selection tools for lower PAC, and estimating inheritance of drought resistance and aflatoxin traits in peanut. The first study (Chapter III) was focused on the association between root characteristics of peanut grown in hydroponic and pot experiments in order to explore the easiest way to study root characteristics of peanut. The second (Chapter IV) examined the association between surrogate traits of drought tolerance and aflatoxin contamination in peanut genotypes under terminal drought, the third and forth part (Chapter V and VI) estimated the heritabilities of terminal drought resistance traits, agronomic traits, and aflatoxin traits, and estimate genotypic and phenotypic correlations between drought-resistant traits and agronomic traits and PAC in peanut under terminal drought. The results of each study are presented individually in Chapters III to VI and were discussed separately. In this chapter, the main findings of our study will be discussed.

Chapter III clearly demonstrated that root characteristics of peanut grown in hydroponic were closely related with those of peanut grown in both small and large pot conditions. In general, the performance of peanut genotypes with respect to root characteristics in pot conditions was quite similar to that of peanut genotypes grown in hydroponic. Root dry weight, root length, root surface, average diameter of roots, and root volume of peanut in hydroponic were positively correlated with root characteristics in pot studies. This indicates that the hydroponic systems could be used to select peanut with difference root characteristics, such as root dry weight, root length, root average diameter, and root volume instead of selection of these characteristics in peanut grown in soil medium. Similar results have been observed when this issue was investigated using other field crops (Mian et al., 1993; Ogbonnaya et al., 2003). Mian et al., (1993) found correlations between root and shoot fresh weight in hydroponic for wheat grown in containers with adequate or excess moisture. Their studies indicated that the root and shoot growth of wheat in hydroponic culture were to some extent predictive of root and shoot growth in soil medium. Ogbonnaya et al., (2003) also found that the correlation between root volume of cowpea in hydroponic and pot conditions was significant.

The largest root production of peanut genotypes grown in hydroponic conditions were Virginia-types followed by Spanish-types. Tifton-8 and KK 60-3 had consistently higher values for all root characteristics compared to the other genotypes. ICGV 98300, ICGV 98324, ICGV 98330, ICGV 98348, and non-nod exhibited poorly for all characteristics. These results were consistent with those of Ketring (1984) and Rucker et al., (1995) who investigated the root diversity among different peanut genotypes in soil medium. In our study, the growth of root characteristics showed typical sigmoid curves of plant growth in which the curves reached a plateau between 60-100 DAP and then declined at 120 DAP. In a hydroponic study, Pandey and Pendleton (1986) found substantial genetic variation and also demonstrated that root length and root volume of peanut increased exponentially up to 70 DAP. These results confirm those reported earlier by McCloud (1974) who found a maximum accumulation of dry weight for peanut root systems by 78 DAP. Meisner and Karnok

(1992) found that root growth of peanut in soil medium can continue until 110 days after planting. In this study, the assessment of root at 80 DAT was the best because of high F-ratios and low CVs.

Literature showed that hydroponic culture can be used for screening cultivars with improved drought tolerance. Ogbonnaya et al., (2003) found significant relationships between drought-resistant traits (WUE) of cowpea in field conditions and root biomass, root volume, and shoot biomass in hydroponic. Furthermore, Ekanayake et al., (1985) observed that root characteristics of rice grown in hydroponic culture were significantly correlated with visual field drought resistance scores and with leaf water potential.

Drought stress can be simulated under hydroponic conditions, and induced by adding suitable osmotic substance. This might be a useful tool to study the effect of drought in field crops. Fan and Blake (1997) studied the use of polyethylene glycol (PEG), an osmotic substance with a high molecular weight, to simulate drought. They observed that when PEG contacts with leaves, it can damage leaf tissue and stomata. Mannitol is an alternative osmotic substance that could be used to induce water stress without directly damaging leaf tissue (Dastgheib et al., 1990). However, additional research on hydroponic culture with simulated drought is needed before using it to study the effect of drought in peanut.

In Chapter IV, our findings indicated that drought promoted the growth and persistence of *A. flavus* populations. These are in agreement with the reports by Blankenship et al., (1984) who also observed that *A. flavus* grows very readily under high soil temperatures and low soil moisture contents. Our results reconfirm the idea that high soil temperature and terminal drought conditions are favorable for seed colonization by *A. flavus* and consequent aflatoxin contamination. Drought-resistant genotypes seemed to have lower *A. flavus* colonization and aflatoxin contamination. Tifton-8 which is a drought resistant germplasm line had low seed colonization and aflatoxin contamination. These results confirm those reported earlier 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 under drought. We also found that the ICRISAT genotypes ICGV 98305, ICGV 98348, and ICGV 98353 also had relatively low aflatoxin contamination.

Reponses of drought on the basis of yield and biomass were investigated in Chapter IV. The results demonstrated that terminal drought had more effect on pod yield than on total biomass. With regard 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. Tifton 8 and KK60-3 exhibited the highest biomass production under terminal drought. Drought resistance germplasm line, IGCV 98348, had the highest pod yield under water stress. Drought-tolerant genotypes on basis of high DTI of total biomass and pod yield were ICGV 98305, ICGV 98324, and ICGV 98348.

Field-based selection approaches for eliminating PAC have been slow due to large and uncontrollable environmental effects and G x E interactions, leading to large coefficients of variation in aflatoxin concentrations (Anderson et al., 1995; Holbrook et al., 1994). In Chapter IV, the correlations between physiological traits for drought resistance and *A. flavus* colonization and PAC were consistent even though the ranking of cultivars in *A. flavus* infection and PAC was quite different between the years. This indicated that breeding for low PAC in peanut might be achieved based on physiological selection for drought resistance. Correlations between *A. flavus* colonization and PAC and surrogate traits of drought resistance were found only under water stressed conditions but were not found under well watered conditions. Correlations between DTI of biomass and PAC revealed that peanut genotypes with an ability to maintain high biomass production under terminal drought also had relatively low PAC. Positive correlations between SLA, DSR, and canopy temperature and PAC were also significant. Correlation between DSR and aflatoxin production has been reported 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 aflatoxin contamination and yield under drought-stress conditions. Negative and significant correlations between ChlD and RWC and PAC have also been observed in this study. These observations indicated that a genotype which had a greater level of drought tolerance would be more resistant to PAC than a drought susceptible genotype.

Results presented in Chapter IV indicated that SLA and RWC may be useful and promising tools for selecting peanut genotypes with reduced *A. flavus* colonization and PAC. SLA has been used to identify drought-resistant traits of peanut, and can be an efficient tool for selecting peanut with drought tolerance in breeding programs (Nageswara Rao and Wrigth 1994; Wright et al., 1994). SLA and RWC are less variable and cheaper to measure than PAC. 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) found the relationship between SLA and RWC was conclusive evidence for the role of SLA in maintaining plant water status during stress and demonstrated the ability of genotypes with low SLA to maintain leaf water status to support metabolic activities as well as to maintain favorable leaf temperature. The ability to maintain pod and plant moisture contents under drought stress has been proposed as a main mechanism that can help to maintain the capacity of plants to produce stilbene phytoalexin preventing PAC (Dorner et al., 1989; Wotton and Strange, 1985). Hence, a possible means of reducing PAC in peanut is the use of cultivars with improved resistance to drought stress.

Chapter V showed that heritability estimates for physiological traits (HI, SCMR, and SLA) were higher than for agronomic traits, and varied among crosses. The estimates of high heritability for physiological traits in the present study were generally in agreement with those previously reported by Songsri et al., (2008). Ntare and Williams, (1998) also reported that heritability of pod yield was lower than partitioning coefficient but higher than other physiological components (crop growth rate and duration of reproduction growth) of their yield model. Cruickshank et al., (2004) also found that heritability estimates for partitioning factor like HI were high (varied from 58-85 %) and varied significantly between crosses depending on levels of genetic variation in parents. Thus, the expected genetic gain per cycle of selection will be less for pod yield and biomass compared with HI, SCMR, and SLA because they were found to have low heritability.

The large heritability for HI and for SCMR and SLA indicates that selection for these traits should be very effective. Additive gene action has been the main factor responsible for variation in many agronomic traits in peanut. Previous studies reported that HI and SLA are mainly under additive genetic control and SCMR was found to be under the influence of both additive and non additive gene effects (Dwivedi et al., 1998; Jayalakshmi et al., 1999; Lal et al., 2006; Nigam et al., 2001; Suriharn et al., 2005). Hence, selection should be effective. Nigam et al., (2001) found that the selection for SLA and HI can be effective in early generations. They also suggestd that the selection can be done in late generations to exploit the effect of additive x additive interaction.

Genetic correlations between physiological traits and economic traits can predict the response of yield and other agronomic traits from selection on the basis of the physiological traits. Genotypic associations in our study (Chapter V) demonstrated that lower SLA and higher HI and SCMR were associated with increased pod yield. Genotypic correlations between SCMR and SLA and agronomic traits were weak and found to be lower than r_G between HI and agronomic traits. However, SCMR and SLA are markedly less costly to evaluate and have been used to identify drought resistance in peanut. Although SLA is affected by environment and genotype, the relationship between SLA and Δ and WUE is apparently consistent across environments in peanut (Nageswara Rao and Wright, 1994; Wright et al., 1994; Upadhyaya, 2005). Furthermore, SLA was also found to be closely associated with HI (Songsri et al., 2008) and SCMR, a rapid assessment for leaf nitrogen and chlorophyll content in peanut (Nageswara Rao et al., 2001; Songsri et al., 2008; Upadhyaya, 2005).

Because of low r_G between SCMR and SLA and agronomic traits found in Chapter V, the use of a combination of physiological traits as a selection index may be advantageous to increase the effectiveness of drought resistance breeding programs. In addition, Bandyopadhyay et al., (1985) and Subbarao et al., (1995) suggested that breeding for drought resistance, using an integrated selection index based on physiological traits such as leaf area, specific leaf weight and leaf dry weight and components of yield was more efficient than an index based on yield components alone, and are more useful in crop improvement programs than single traits.

G x E interactions of aflatoxin traits found in this study confirmed that field-based selection approaches for eliminating PAC in peanut will be less successful.

Moreover, heritabilities for seed infection and aflatoxin contamination were low to moderate (Chapter VI). Hence, physiological traits for drought resistance are promising traits to reduced PAC in peanut. Genotypic associations between aflatoxin traits and drought-tolerant traits found in this study (Chapter VI) demonstrated that genotypes with high DTI (PY), DTI (BIO), and HI tend to have low *A. flavus* infection and PAC. This implied that the ability to maintain higher biomass and pod yield during drought periods may be important traits enabling cultivars to resist aflatoxin production. Weak correlations between PAC and pod yield in this study confirmed the finding of Holbrook et al., (2000) who found a negative phenotypic correlation between aflatoxin contamination and yield under drought stressed conditions. Hence, selection of genotypes which have higher yield under drought conditions could also lower aflatoxin contamination compared to lower yielding genotypes.

Researchers have demonstrated the genotypic associations between drought-tolerant traits and PAC in order to identify an indirect selection tool for eliminating PAC in peanut. Arunyanark et al., (2009a) found that PAC was associated well with SLA, SCMR, root length density, and drought tolerance indices under long period drought conditions. SLA and SCMR seem to be the best indirect selection criteria for reducing PAC because these traits have high heritability, and are less variable and less expensive to measure (Songsri et al., 2008). Close genotypic associations between physiological traits for drought resistance and aflatoxin traits reported in Chapter VI suggested that SLA, and SCMR are potentially useful as indirect selection tools to reduce PAC. In this study, we have found that selection for high SCMR and low SLA is expected to have a greater effect on lower *A. flavus* infection and PAC than selection for the other drought-resistant traits. Hence, these traits should be more applicable in breeding programs with large segregating populations.

In conclusion, drought-tolerant traits can be potentially used as indirect selection tools for resistance to PAC. Our findings showed that genotypes with high chlorophyll density, high water status in leaf, and low SLA or high leaf thickness under terminal drought had relatively low *A. flavus* colonization and PAC. These physiological traits can also be used as effective tools for selection of peanut genotypes with terminal drought tolerance and low levels of PAC. SLA seems to be the best surrogate trait for drought tolerance and low PAC because its low G x E interactions and a good correlation to PAC. The genotypes ICGV 98305, ICGV 98348, and ICGV 98353 that are elite drought-resistant lines from ICRISAT, and Tifton- 8 that is a drought-resistant line from USDA were observed to have relatively low preharvest aflatoxin contamination in these studies. Inheritant study (Chapter V and VI) revealed that physiological traits are potentially useful as an indirect selection index for terminal drought resistance and lower PAC because of their low G x E interactions, high heritabilities and significant correlations with agronomic traits and aflatoxin traits. Plant breeding approaches on the basis of surrogate traits for drought resistance might be effective for improving terminal drought tolerance and lower PAC in peanut. This study found that selection for HI is expected to have a greater effect on yield and other agronomic traits than selection for SCMR and SLA. However, SCMR and SLA are easier to measure and should be more applicable in breeding programs with large segregating populations. Furthermore, SCMR and SLA were also correlated well with PAC, and the correlations with PAC were stronger than with HI. The use of an integrated selection index should be useful to increase the effectiveness of breeding for drought resistance and lower PAC.

REFERENCES

Abdollahi, A., and R.L. Buchanan. 1981. Regulation of aflatoxin biosynthesis – induction of aflatoxin production by various carbohydrates. Journal of Food Science 46: 633–635.

Akkasaeng, C., N. Vorasoot, S. Jogloy, and A. Patanotai. 2003. Relationship between SPAD readings and chlorophyll contents in leaves of peanut (*Arachis hypogaea* L.). Thai Journal Agricultural Science 36(3): 279–284.

Anderson, W. F., C. C. Holbrook, and D. M. Wilson. 1996. Development of greenhouse screening for resistance to *Aspergillus parasiticus* infection and preharvest aflatoxin contamination in peanut. Mycopathologia 135: 115-118.

Anderson, W. F., C. C. Holbrook, D. M. Wilson, and M. E. Matheron. 1995. Evaluation of preharvest aflatoxin contamination in several potentially resistant peanut genotypes. Peanut Science 22: 29-32.

Araus, J.L., G.A. Slafer, M.P. Reynolds, and C. Royo. 2002. Plant breeding and drought in C3 cereals: what should we breed for?. Annals of Botany 89: 925–940.

Arunyanark, A., S. Jogloy, C. Akkasaeng, N. Vorasoot, T. Kesmala, R.C. Nageswara Rao, G.C. Wright, and A. Patanothai. 2008. Chlorophyll stability is an indicator of drought tolerance in peanut. Journal of Agronomy and Crop Science 194: 113–125.

Arunyanark, A., S. Jogloy, S. Wongkaew, C. Akkasaeng, N. Vorasoot, G.C. Wright, Rao C.N. Rachaputi, and A. Patanothai. 2009a. Association between aflatoxin contamination and drought tolerance traits in peanut. Field Crop Research 114: 14-22.

Arunyanark, A., S Jogloy, S. Wongkaew, C Akkasaeng, N. Vorasoot, T. Kesmala, and A. Patanothai. 2009b. Heritability of aflatoxin resistance traits and correlation of aflatoxin resistance and drought tolerance traits under drought conditions in peanut. Field Crop Research (In press)

Aujla, S. S., J. S. Chohan, and V. K. Mehan. 1978. The screening of peanut varieties for the accumulation of aflatoxin and their relative reaction to the toxigenic isolate of *Aspergillus flavus* Link ex Fries. Journal of Research Punjab Agricultural University 15: 400-403.

Awal, M.A., and T. Ikeda. 2002. Recovery strategy following the imposition of episodic soil moisture deficit stands of peanut (*Arachis hypogaea* L). Journal of Agronomy and Crop Science 188: 185–192.

Azaizeh, H. A., and R. E. Pettit. 1986. Screening peanut genotypes (*Arachis hypogaea* L) for resistance to *Aspergillus flavus* group of fungi and aflatoxin production. Phytopathology 76: 1091.

Azaizeh, H. A., and R. E. Pettit. 1987. Influence of tannin –related compounds from peanut seed coats and cotyledons on *Aspergillus parasiticus* growth and aflatoxin production. Phytopathology 77: 1703.

Azaizeh, H. A., R. E. Pettit, O. D. Smith, and R. A. Taber. 1989. Reaction of peanut genotypes under drought stress to *Aspergillus flavus* and *A. parasiticus*. Peanut Science 16: 109–113.

Bandyopadhyay, A., V. Arunachalan, and K. Venkajah. 1985. Efficient selection intensity in early generation index selection in groundnut (*Arachis hypogaea* L.). Theoretical and Applied Genetics 71: 300 - 304.

Bartz, J.A., A.J. Norden, J.C. LaPrade, and T.J. DeMuynk. 1978. Seed tolerance in peanuts (*Arachis hypogaea* L) to members of *Aspergillus flavus* group fungi. Peanut Science 5: 53–56.

Basha, S.M. 1992. Soluable composition of peanut seed. Journal of Agricultural and Food Chemistry 40: 780–783.

Bennett, J.W., and M. Klich. 2003. Mycotoxins. Clinical Microbiology Reviews 16: 497–516.

Beuchat, L.R., C.T. Young, and J.P. Cherry. 1975. Electrophoretic patterns and free amino acid composition of peanut meal fermented with fungi. Canadian Institute of Food Science and Technology 8: 40–45.

Bewley, J. D. 1979. Physiological aspects of desiccation tolerance. Annual Review of Plant Physiology 30:195-238.a

Bhalani, G.K. and M. Parameswaran. 1992. Influence of differential irrigation on kernel lipid profile in groundnut. Plant Physiology and Biochemistry19: 11-14.

Bindu Madhava, H., M.S. Sheshshayee, A.G. Shankar, T.G. Prasad, and M. Udayakumar. 2003. Use of SPAD chlorophyll meter to assess transpiration efficiency of peanut, pp. 3–9. *In* A.W. Cruickshank, N.C. Rachaputi, G.C. Wright, and S.N. Nigam (eds.), Breeding for drought–resistant peanuts. 25–27 February 2002. ICRISAT Centre, Andhra Pradesh, India.

Blad, B.L., B.R. Gardner, D.G. Watts, and N.J. Rosenberg. 1980. Remote sensing of crop moisture status. p. 1-26. *In* Remotely sensed crops temperature for water resources management. Agriculture Meteorology Progress Report. University of Nebraska, Lincoln, Nebraska, USA.

Blankenship, P.D., R.J. Cole, T.H. Sanders, and R.A. Hill. 1984. Effect of geocarposphere temperature on pre–harvest colonization of drought–stressed peanuts by *Aspergillus flavus* and subsequent aflatoxin contamination. Mycopathologia 85: 69–74.

Blum, A. 1988. Plant breeding for stressed environments. CRC Press, Boca Raton, Florida.

Blum, A., and C.Y. Sullivan. 1986. The comparative drought resistance of landraces of sorghum and millet from dry and humid regions. Annals of Botany 57: 835-840.

Boote, K. J. 1982. Growth stage of peanut (*Arachis hypogaea* L.). Peanut Science 9: 35-40.

Boyer, J. S. 1983. Subcellular mechanisms of plant response to low water potential. Agricultural Water Management 7: 239-248.

Branch, W.D., and C.K. Kvien. 1992. Peanut breeding for drought resistance. Peanut Science 19: 44–46.

Bricke, A.A. 1989. MSTAT-C user's guide. Michigan State University, East Lansing, Michigan.

Buchanan, R.L., and D.F. Lewis. 1984a. Caffeine inhibition of aflatoxin synthesis: probable site of action. Applied and Environmental Microbiology 47: 1216–1220.

Buchanan, R.L., and H.G. Stahl. 1984b. Ability of various carbon–sources to induce and support aflatoxin synthesis by *Aspergillus parasiticus*. Journal of Food Safety 6: 271–279.

Burow, G.B., T.C. Nesbitt, J. Dunlap, and N.P. Keller. 1997. Seed lipoxygenase products modulate *Aspergillus* mycotoxin biosynthesis. Molecular Plant–Microbe Interactions 10: 380–387.

Calvo, A.L., H.W. Hinze, H.W. Gardner, and N.P. Keller. 1999. Sporogenic effect of polyunstaurated fatty acids on *Aspergillus* spp development. Applied and Environmental Microbilogy 65: 3668–3673.

Chapman, S.C., M.M. Ludlow, and F.P.C. Blamey. 1993a. Effect of drought during early reproductive development on the dynamics of yield development of genotypes of peanut (*Arachis hypogaea* L.). Field Crops Research 32: 227–242.

Chapman, S.C., M.M. Ludlow, F.P.C. Blamey, and K.S. Fisher. 1993b. Effect of drought during early reproductive development on growth of cultivars of groundnut (*Arachis hypogaea* L.). I. Utilization of radiation and water during drought. Field Crops Research 32: 193–210.

Chapman, S.C., M.M. Ludlow, F.P.C. Blamey, and K.S. Fischer. 1993c. Effect of drought during early reproductive development on the dynamics of yield development of genotypes of peanut (*Arachis hypogaea* L.) II. Biomass production, pod development and yield. Field Crops Research 32: 211–225.

Chenault, K.D., H.A. Melouk, and C.C. Holbrook. 2004. Post-harvest aflatoxin accumulation in transgenic peanut lines containing anti-fungal genes. Phytopathology 94: S18.

Chu, F.S. 1989. Current immunochemical methods for analysis of aflatoxin in groundnut and ground products; aflatoxin contamination of groundnut, pp. 161–172. *In* D. McDonald, V.K. Mehan, and S.D. Hall (eds.), Proceedings of the International Workshop in Aflatoxin Contamination of Groundnuts. 6–9 Oct. 1987. ICRISAT center, Patancheru, Andhra Pradesh, India.

Chu, F.S., T.S.L. Fan, G.S. Zhang, and Y.C. Xu. 1987. Improved enzyme-linked immunosorbent assay for aflatoxin B1 agricultural commodities. Journal of the Association of Official Analytical Chemists 70: 1125–1128.

Clavel, D., B. Sarr, E. Marone, and R. Ortiz. 2004. Potential agronomic and physiological traits of Spanish groundnut varieties (*Arachis hypogaea* L.) as selection criteria under end-of cycle drought conditions. Agronomie 24: 101-111.

Clavel, D, O. Diouf, J. L. Khalfaoui, and S. Braconnier. 2006. Genotypes variations in fluorescence parameters among closely related groundnut (*Arachis hypogaea* L.) lines and their potential for drought screening programs. Crop Science 34: 92–97.

Coffelt, T. A., R. O. Hammons, W. D. Branch, R. W. Mozingo, P. M. Phipps, J. C. Smith, R. E. Lynch, C. S. Kvien, D. L. Ketring, D. M. Porter, and A. C. Mixon. 1985. Registration of Tifton-8 peanut germplasm. Crop Science 25: 203.

Cole, R.J., T.H. Sanders, J.W. Dorner, and P.D. Blankenship. 1989. Environmental conditions required to induce preharvest aflatoxin contamination of groundnuts: summary of six year's research, pp. 279–287. *In* Aflatoxin contamination groundnut. Proceedings of the International Workshop. ICRISAT Centre. Patancheru, Andhra Pradesh, India.

Cole, R.J., T.H. Sanders, R.A. Hill, and P.D. Blankenship. 1985. Mean geocarposphere temperatures that induce preharvest aflatoxin contamination of peanuts under drought stress. Mycopathologia 91: 41–46.

Cole, R. J., V. S. Sobolev, and J. W. Dorner. 1993. Potentially important sources of resistance to prevention of preharvest aflatoxin contamination in peanuts. Vol. 25 pp. 78. *In* Proceedings in American Peanut Research and Education Society, Huntsville, Alabama, USA.

Collino, D.J., J.L. Dardanelli, R. Sereno, and R.W. Racca. 2001. Physiological responses of argentine peanut varieties to water stress. Light interception, radiation use efficiency and partitioning of assimilate. Field Crop Research 68: 133–142.

Conkerton, E.J., L.F. Ross, D.J. Daigle, C.S. Kvien and C. McCombs. 1989. The effect of drought stress on peanut seed composition. II. Oil, protein and minerals. Oleagineux 44: 593-599.

Costa, C., L.M. Dwyer, X. Zhou, P. Dutilleul, C. Hamel, L.M. Reid, and D.L. Smith. 2002. Root morphology of contrasting maize genotypes. Agronomy Journal 94: 96-101.

Craufurd, P. Q., T. R. Wheeler, R. H. Ellis, R. J. Summerfield, and J. H. Williams. 1999. Effect of temperature and water deficit on water-use efficiency, carbon isotope discrimination, and specific leaf area in peanut. Crop Science 39: 136-142.

Cruickshank, A.L., A. Dowkiw, G.C. Wright, R.C. Nageswara Rao, and S.N. Nigam. 2004. Heritability of drought–resistance traits in peanut. *In* T. Fischer, N. Turner, J. Angus, L. McIntyre, M. Robertson, A. Borrell, and D. Lloyd (eds.), New directions for a diverse planet. Proceedings for the 4th International Crop Science Congress. 26 September – 1 October 2004. Brisbane, Australia.

Cruickshank, A.L., G.C. Wright, and R.C. Nageswara Rao. 2000. "Streeton" An aflatoxin tolerant peanut cultivar for the Australian Peanut Industry, p. 27. In American Peanut Research and Education Society Conference. Point clear, Alabama, USA.

Del Rosario, D.A., and F.F. Fajado. 1988. Morphophysiological responses of ten peanut (*Arachis hypogaea* L.) varieties to drought stress. The Philippine Agriculturist 71: 447–459.

Doehlert, D.C., D.T. Wicklow, and H.W. Gardner. 1993. Evidence implicating the lipoxygenase pathway in providing resistance to soybeans against *Aspergillus flavus*. Phytopathology 83: 1473–1477.

Doorenbos, J., and W.O. Pruitt. 1992. Calculation of crop water requirements, pp. 1–65. *In* FAO Irrigation and Drainage Paper No: 24. FAO of the United Nation. Rome, Italy.

Dorner, J.W., R.J. Cole, T.H. Sanders, and P.D. Blankenship. 1989. Interrelationship of kernel water activity, soil–temperature, maturity, and phytoalexin production in preharvest aflatoxin contamination of drought–stressed peanuts. Mycopathologia 105: 117–128.

Diener, U. L., R. J. Cole, T. H. Sanders, G. A. Payne, L. S. Lee, and M. A. Klich. 1987. Epidemiology of aflatoxin formation by *Aspergillus flavus**. Annual Review of Phytopathology. 25: 249-270.

Duncan, W. G., D. E. McCloud, R. L. McGraw, and K. J. Boote. 1978. Physiological aspects of peanut yield improvement. Crop Science 18: 1015 - 1020.

Dwivedi, S.L., S.N. Nigam, R.C. Nageswa Rao, U. Singh and K.V.S. Rao. 1996. Effect of drought on oil, fatty acids and protein contents of groundnut (*Arachis hypogaea* L.) seeds. Field Crops Research 48: 125–133.

Dwivedi, S. L., S. N. Nigam, S. Chandra, and V. M. Ramraj. 1998. Combining ability of biomass and harvest index under short and long-day conditions in groundnut. Annals of Applied Biology 133: 237 - 244.

Ekanayake, I.J., J.C. O'Toole, D.P. Garrity, and T.M. Masajo. 1985. Inheritance of root characteristics and their relations to drought resistance in rice. Crop Science 25: 927-933.

Ellis, W.O., J.P. Smith, B.K. Simpson, and J.H. Oldham. 1991. Aflatoxins in food: occurrence, biosynthesis, effects on organisms, detection, and methods of control. Critical Reviews in Food Science and Nutrition 30: 403–439.

Epstein, E. 1972. Mineral nutrition of plants, Principal and Perspective. John Wiley & Sons, New York.

Erusha, K.S., R.C. Shearman, T.P. Riordan, and L.A. Wit. 2002. Kentucky bluegrass cultivar root and top growth responses when grown in hydroponics. Crop Science 42: 848-852.

Fabbri, A. A., C. Fanelli, G. Panfili, S. Passi, and P. Fasella. 1983. Lipoperoxidation and aflatoxin biosynthesis by *Aspergillus parasiticus* and *A. flavus*. Journal of General Microbiology 129:3447–3452.

Falconer, D. S., and T. F. C.Mackay. 1996. Introduction to quantitative genetics. Longman, London.

FAOSTAT. 2008. Statistical databases. (cited on Nov. 25, 2008) Available from: URL: http://faostat.fao.org.

Farquhar, G.D., M.H. O'Leary, and J.A. Berry. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Australian Journal Plant Physiology 9: 121–137.

Feng, G.H., and T.J. Leonard. 1995. Characterisation of the polyketide synthase gene (pksLI) required for aflatoxin biosynthesis by *Aspergillus parasiticus*. Journal of Bacteriology 177: 6246–6254.

Fry, W. E. 1982. Principles of Plant Disease Management. Academic Press. New York.

Girdthai, T., S. Jogloy, N. Vorasoot, C. Akkasaeng, S. Wongkaew, C. C. Holbrook, and A. Patanothai. 2010. Associations between physiological traits for drought tolerance and aflatoxin contamination in peanut genotypes under terminal drought. Plant Breeding doi:10.1111/j.1439-0523.2009.01738.x.

Gomez, K.A., and A.A. Gomez. 1984. Statistical procedures for agricultural research. 2nd ed. John Wiley & Sons: New York.

Gqaleni, N., J.E. Smith, J. Lacey, and G. Gettinby. 1997. Effects of temperature, water activity, and incubation time on production of aflatoxins and cyclopiazonic acid by an isolate of *Aspergillus flavus* in surface agar culture. Applied and Environmental Microbiology 63: 1048-1053.

Grimm, D.T., T.H. Sanders, H.E. Pattee, D.E. Williams, and S. Sanchez–Dominguez. 1996. Chemical composition of *Arachis hypogaea* L. Sub sp. hypogaea Var. hirsuta peanuts. Peanut Science 23: 111–116.

Girma, F. S., and D. R. Krieg. 1992. Osmotic adjustment in sorghum: I. Mechanisms of diurnal osmotic potential changes. Plant Physiology 99: 583-588.

Hall, A.J., and C. P. Wild. 2003. Liver cancer in low and middle income countries: prevention should target vaccination, contaminated needles and aflatoxins. British Medical Journal 326, 994–995.

Hanks, R.J., J. Keller, V.P. Rasmussen, and G.D. Wilson. 1976. Line–source sprinkler for continuous variable irrigation crops production studies. Soil Science Society of America Journal 40: 426–429.

Hebbar, K.B., V.R. Sashidhar, M. Udayakumar, R. Devendra, and R.C. Nageswara Rao. 1994. A comparative assessment of water use efficiency in groundnut (*Arachis hypogaea*) grown in containers and in the field under water–limited conditions. Journal of Agricultural Science 122: 429–434.

Hill, R.A., P. D. Blankenship, R.J. Cole, and T.H. Sanders. 1983. Effects of soil–moisture and temperature on preharvest invasion of peanuts by the *Aspergillus flavus* group and subsequent aflatoxin development. Applied and Environmental Microbiology 45: 628–633.

Hill, W.A., D.G. Mortley, C.L. Mackoiak, P.A. Loretan, T.W. Tibbitts, R.M. Wheeler, C.K. Bonsi, and C.E.Morris. 1992. Growing root, tuber and nut crops hydroponically for cells. Advances in Space Research 12: 125-131.

Holbrook, C.C., and H.T. Stalker. 2003. Peanut breeding and genetic resources. Plant Breeding Reviews 22: 297–356.

Holbrook, C. C., B. Z. Guo, D. M. Wilson, and P. Timper. 2009. The U.S. breeding program to develop peanut with drought tolerance and reduced aflatoxin contamination. Peanut Science 36: 50-53.

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

Holbrook, C. C., D. M. Wilson, M. E. Matheron, J. E. Hunter, D. A. Knauft, and D. W. Gorbet. 2000b. *Aspergillus* colonization and aflatoxin contamination in peanut genotypes with reduced linoleic acid composition. Plant Disease 84:148–150.

Holbrook, C.C., M. E. Matheron, D.W. Wilson, W.F. Anderson, M. E. Will, and A. J. Noden. 1994. Development of a large-scale field screening system for resistance to preharvest aflatoxin contamination. Peanut Science 21: 20-22.

Holbrook, C. C., P. Ozias-Akins, P. Timper, D. M. Wilson, E. Cantonwine, B. Z. Guo, D. G. Sullivan, and W. Dong. 2008. Research from the coastal plain experiment station, Tifton, Georgia to minimize aflatoxin contamination in peanut. Toxin Reviews 27: 391-410.

Holland, J. B. 2001. Epistasis and plant breeding. Plant Breeding Reviews 21: 27 - 92.

Holland, J. B., W. E. Nyquist, and C. T. Cervartes-Martinez. 2003. Estimating and interpreting heritability for plant breeding: an update. Plant Breeding Reviews 22: 2 - 112.

Horn, B.W. 2005. Colonisation of wounded peanut seeds by soil fungi: Selectivity for species from *Aspergillus* section *Flavi*. Mycologia 97: 202–217.

Hsiao, T.C., J.C. O'Toole, E.B. Yambao, and N. C. Turner. 1984. Influence of osmotic adjustment on leaf rolling and tissue death in rice (*Oryza sativa* L.). Plant Physiology 75: 338–341.

Hubick, K.T., G.D. Farquhar, and R. Shorter. 1986. Correlation between water–use efficiency and carbon isotope discrimination in diverse peanut (*Arachis*) germplasm. Australian Journal Plant Physiology 13: 803–816.

Hubick, K.T., R. Shorter, and G.D. Farquhar. 1988. Heritability and genotype x environment interaction of carbon isotope discrimination efficiency in peanut (*Arachis hypogaea* L.). Australian Journal Plant Physiology 15: 799–813.

Ingram, K.T., and G.A. Leers. 2001. Software for measuring root characteristics from digital images. Agronomy Journal 93: 918-922.

Ingram, K.T., G.F. Patena, and C.C. Holbrook. 1999. Drought and temperature effect on aflatoxin resistance peanut. Paper presented to 1999 Multicrop Aflatoxin Elimination Workshop. 20– 22 October 1999. Atlanta, Georgia, USA.

Isleib, T.G. and J. C. Wynne. 1992. Use of plant introductions in peanut improvement. *In*: Use of plant introductions in cultivar development. Madison: Crop Science Society of America 2: 75-116.

Isleib, T. G., C. C. Holbrook, and D. W. Gorbet. 2001. Use of peanut introductions in peanut cultivar development. Peanut Science 28:96-113.

Jackson, P., M. Roberston, M. Cooper, and G. Hammer. 1996. The role of physiological understanding in plant breeding; from a breeding perspective. Field Crop Research 49: 11–39.

Jayalakshmi, V., C. Rajareddy, P. V. Reddy, and R. C. Nageswara Rao. 1999. Genetic analysis of carbon isotope discrimination and specific leaf area in groundnut (*Arachis hypogaea* L.). Journal of Oilseeds Research 16: 1 - 5.

Johensen, C., B. Baldev, J. B. Brouwer, W. Erskine, W. A. Jermyn, L. J. Lang, B. A. Malik, A. A. Miah, and S. N. Silim. 1994. Biotic and a biotic stress containing productivity of cool season legume in Asia, Africa and Oceania. pp. 175-194, *In* Muehlbauer, F. J. and W. J. Kaiser (eds). Expanding the production and Use of Cool Season Food Legume. Kluwer, Academic Publishers, Dordrecht, the Netherlands.

Jones, R. K., H. E. Duncan, G. A. Payne, and K.G. Leonard. 1980. Factors influencing colonization by *Aspergillus flavus* in silk-inoculated corn. Plant Disease 64: 859-863.

Jongrungklang, N., B. Toomsan, N. Vorasoot, S. Jogloy, T. Kesmala, and A. Patanothai. 2008. Identification of peanut genotypes with high water use efficiency under drought stress conditions from peanut germplasm of diverse origins. Asian Journal of Plant Science 7: 628–638.

Jordan, W.R., F.R. Miller, and D.E. Morris. 1979. Genetic variation in root and shoot growth of sorghum in hydroponics. Crop Science 19: 468-472.

Jordan, W.R., W. A. Dugas, and P. J. Shouse. 1983. Strategies for crop improvement for drought prone regions. Agricultural Water Management 7: 281-299.

Karnok, K.J., and R.T. Kucharski. 1982. Design and construction of a rhizotron-lysimeter facility at the Ohio State University. Agronomy Journal 74:152-156.

Ketring, D.L. 1984. Root diversity among peanut genotypes. Crop Science 24: 229-232.

Kisyombe, C.T., M.K., Beute, and G.A. Payne. 1985. Field evaluation of peanut enotypes for resistance to infection by *Aspergillus parasiticus*. Peanut Science 12: 12–17.

Klich, M.A., L.H. Tiffany, and G. Knaphus. 1992. Ecology of the *Aspergilli* of soils and litter, pp. 329–354. *In* J.W. Bennett, and M.A. Klich (eds.), *Aspergillus*: biology and industrial applications. Butterworth Heineman: Boston.

Knauft, D. A., and D. W. Gorbet. 1989. Genetic diversity among peanut cultivars. Crop Science 29: 1417-1422.

Kramer, P. J. 1980. Drought stress and the origin of adaptation. pp. 7–20 *In* N. C. Turner, and P. J. Kramer, (eds.), Adaptation of Plant to Water and High Temperature Stress, John Wiley & Sons, New York.

Krapovickas, A., and W. C. Gregory. 1994. Taxonomy of the genus *Arachis* (Leguminosae). Bonplanda 8:1-186.

Kushalappa, A. C., Jerry A. Bartz and J. Norden. 1979. Susceptibility of pods of different peanut genotypes. Physiopathology. 69:159-162.

Lal, C., K. Hariprasanna, A. L. Rathnakumar, H. K. Gor, and B. M. Chikani. 2006. Gene action for surrogate traits of water-use efficiency and harvest index in peanut (*Arachis hypogaea*). Annals of Applied Biology 148: 165 - 172.

Lancaster, M. C., F.P. Jenkins, and J.M. Philip. 1961. Toxicity associated with certain samples of groundnuts. Nature (London) 192:1095–1096.

Latha, P., P. Sudhakar, Y. Sreenivasulu, P.H. Naidu, and P.V. Reddy. 2007. Relationship between total phenols and aflatoxin production of peanut genotypes under end–of–season drought conditions. Acta Physiologiae Plantarum 29:563–566.

Ludlow, M. M. 1980. Adaptive significance of stomatal responses to water stress. pp 123-138, *In* N. C. Turner and P. J. Kramer, (eds). Adaptation of plants to water and high temperature stress. Wiley. New York.

Lynch, R.E., and D.M. Wilson. 1991. Enhanced infection of peanut, *Arachis hypogaea* L, seeds with *Aspergillus flavus* group fungi due to external scarification of peanut pods by the lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller). Peanut Science 18(2): 110–116.

Lynch, R.E., D.M. Wilson, B.W. Maw. 1990. Enhanced aflatoxin contamination of peanut as a result of insect damage to pods. Proceedings of American Peanut Research and Education Society 22: 78.

Maiti, R.K., P. Wesche–Ebeling, A. Núnez–Gonzalez, and E. Sánchez–Arreola. 2002. Root system and mineral nutrition, pp. 125–146. *In* R.K. Maiti, and P. Wesche–Ebeling (eds.), The peanut (*Arachis hypogaea*) crop. Science Publishers, Inc.: New Hampshire.

Marshall, H.F., L.F. Ross, E.J. Conkerton, D.C. Chapital, and C.K. Kvien. 1991. Effect of diniconazole on the free amino acids and carbohydrates of peanut, *Arachis hypogaea* L. Oléagineux 46: 329–332.

Mathews, R.B., D. Harris, R.C. Nageswara Rao, J.H. Williams, and K.D.R. Wadia. 1988. The physiological basis for yield differences between four genotypes of groundnut (*Arachis hypogaea* L.) in response to drought. I. Dry matter production and water use. Experimental Agriculture 24: 191–202.

McDonald, D., and C. Harkness. 1967. Aflatoxin in groundnut crop at harvest in Northern Nigeria. Tropical Science IX 3: 148–161.

McCloud, D.E. 1974. Growth analysis of high yielding peanuts. Proceedings of Soil and Crop Science Society of Florida. 33: 24-26.

Mehan, V.K. 1989. Screening groundnuts for resistance to seed invasion by *Aspergillus flavus* and to aflatoxin production, pp. 323–334. *In* D. McDonald and V.K. Mehan (eds.), Aflatoxin Contamination of Groundnut. Proceedings of International Workshop, 6–9 October 1987. ICRISAT Centre. Patancheru, Andhra Pradesh, India.

Mehan, V. K., D. McDonald, and K. Rajagopalan. 1987. Resistance of peanut genotypes to seed infection by *Aspergillus flavus* in field trails in India. Peanut Science 14: 17-21.

Mehan, V.K., D. McDonald, N. Ramakrishana, and J.H. Williams. 1986. Effects of genotype and date of harvest on infection of peanut seed by *Aspergillus flavus* and subsequent contamination with aflatoxin. Peanut Science 13: 46–50.

Meisner, C.A., and K.J. Karnok. 1992. Peanut root response to drought stress. Agronomy Journal 84: 159–165.

Merrill, S.D., and D.R. Upchurch. 1994. Converting root numbers observed at minirhizotrons to equivalent root length density. Soil Science Society of America Journal 58: 1061–1067.

Mian, M.A.R., E.D. Nafziger, F.L. Kolb, and R.H. Teyker. 1993. Root growth of wheat genotypes in hydroponic culture and in the greenhouse under different soil moisture regimes. Crop Science 33: 283-286.

Mixon, A. C. 1976. Peanut breeding strategy to minimize aflatoxin contamination. Journal of the American Peanut Research and Education Association 8: 54-58.

Mixon, A. C. 1979. Developing groundnut lines with resistance to seed colonization by toxin–production stains of *Aspergillus* species. PANS USA 25: 394–400.

Mixon, A. C. 1983. Peanut germplasm lines, AR–1, –2, –3, and –4. Crop Science 23: 1021.

Mixon, A. C. 1986. Reducing *Aspergillus* species infection of peanut seed using resistant genotypes. Journal of Environmental Quality 15: 101–103.

Mixon, A. C., and K.M. Rogers. 1973. Peanut accessions resistant to seed infection by *Aspergillus flavus*. Agronomy Journal 65: 560–562.

Mixon, A. C., and K.M. Rogers. 1975. Factors affecting *Aspergillus flavus* Lk ex Fr colonisation of resistant and susceptible genotypes of *Arachis hypogaea* L. Peanut Science 2: 18–22.

Morgan J. M. 1983. Osmoregulation as a selection criterion for drought tolerance in wheat. Australian Journal of Agricultural Research 34: 607-614.

Morgan J. M. 1984. Osmoregulation and water stress in higher plants. Annual Review of Plant Physiology 35: 299-319.

Moran, R. 1981. Formulas for determination of chlorophyll pigment extracted with N, N-Dimethyl formamide. Plant Physiology 69: 1376-1381.

Musingo, M.N., S.M. Basha, T.H. Sanders, R.J. Cole, and P.D. Blankenship. 1989. Effect of drought and temperature stress on peanut (*Arachis hypogaea* L.) seed composition. Journal of Plant Physiology 134: 710-715.

Nageswara Rao, R.C., and G.C. Wright. 1994. Stability of the relationship between specific leaf area and carbon isotope discrimination across environments in peanut. Crop Science 34: 98–103.

Nageswara Rao, R.C., and S.N. Nigam. 2003. Genetic options for drought management in groundnut, pp. 123–141. *In* N.P. Saxena (ed.), Management of agricultural drought: Agronomic and genetic options. Science Publishers, Inc.: New Hampshire.

Nageswara Rao R.C., G.C. Wright and S. Krosch. 2002. Management practices to minimise pre–harvest aflatoxin contamination in Australia peanuts. Australian Journal of Experimental Agriculture 42: 595–605.

Nageswara Rao, R.C., H.S. Talwar, and G.C. Wright. 2001. Rapid assessment of specific leaf area and leaf nitrogen in peanut (*Arachis hypogaea*. L) using chlorophyll meter. Journal of Agronomy and Crop Science 186: 175–182.

Nageswara Rao, R.C., J.H. William, M. Singh. 1989. Genotypic sensitivity to drought and yield potential of peanut. Agronomy Journal 81: 887–893.

Nageswara Rao, R.C., J.H. William, M.V.K. Sivakumar, and K.D.R. Wadia. 1988. Effect of water deficit at different growth phases of peanut. II. Response to drought during pre–flowering phase. Agronomy Journal 80: 431–438.

Nageswara Rao, R.C., L.J. Reddy, V.K. Mehan, S.N. Nigam, and D. McDonald. 1992. Drought research on groundnut at ICRISAT, pp. 455. *In* Nigam S.N. (ed.), Proceedings of the international workshop, Groundnut–a global perspective. 25–29 November 1991. ICRISAT Center, Andhra Pradesh, India.

Nageswara Rao, R.C., M. Udaykumar, G.D. Farquhar, H.S. Talwar, and T.G. Prasad. 1995. Variation in carbon isotope discrimination and its relationship to specific leaf area and ribulose–1, 5–bisphosphate carboxylase content in groundnut genotypes. Australian Journal Plant Physiology 22: 545–551.

Nageswara Rao R.C., Sardar Singh, M.V.K. Sivakumar, K.L.Srivastava, and J.H. Williams. 1985. Effect of water deficit at different growth phase of peanut. I Yield responses. Agronomy Journal 77: 782–786.

Nagrajan, V. and R. V. Bhat. 1973. Ariatoxin production in peanut varieties by *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare. Journal of Applied Microbiology 25: 319-21.

Narasimham, R. L., I. V. S. Rao, and M. Singa Rao. 1977. Effect of moisture stress on response of groundnut to phosphate fertilization. Indian Journal of Agricultural Sciences 47: 573-576.

Nautiyal, P.C., R.C. Nageswara Rao, and Y.C. Joshi. 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 Crops Research 74: 67–79.

Nautiyal, P.C., V. Ravinda, P.V. Zala, and Y.C. Joshi. 1999. Enhancement of yield in peanut following the imposition of transient soil–moisture–deficit stress during the vegetative phase. Experimental Agriculture 35: 371–385.

Nehdi, S. 1989. The geocarposphere mycoflora and resistance of groundnut to *Aspergillus flavus*, pp. 365–378. *In* Aflatoxin contamination of groundnut. ICRISAT Centre. Patancheru, Andhra Pradesh, India.

Ndunguru, B. J., B. R. Ntare, J. H. Williams, and D. C. Greenberg. 1995. Assessment of groundnut cultivars for end-of-season drought tolerance in a Sahelian environment. The Journal of Agricultural Science 125, 79-85.

Nigam, S. N., and R. Aruna. 2008. Stability of soil plant analytical development (SPAD) chlorophyll meter reading (SCMR) and specific leaf area (SLA) and their association across varying soil moisture stress conditions in groundnut (*Arachis hypogaea* L.). Euphytica 160: 111–117.

Nigam, S.N., H.D. Upadhyaya, S. Chandra, R.C. Nageswara Rao, G.C. Wright, and A.G.S. Reddy. 2001. Gene effects for specific leaf area and harvest index in three crosses of groundnut (*Arachis hypogaea*). Annals of Applied Biology 139: 301–306.

Nigam S.N., M.S. Basu, and A.W. Cruickshank. 2003. Hybridization and description of the trait–based and empirical selection programs, pp. 15–17. *In* A.W. Cruickshank, N.C. Rachaputi, G.C. Wright, and S.N. Nigam (eds.), Breeding for drought–resistant peanuts. 25–27 February 2002. ICRISAT Centre. Andhra Pradesh, India.

Nigam, S.N., S. Chandra, K. Rupa Sridevi, A. Manoha Bhukta, G.S. Reddy, R.C. Nageswara Rao, G.C. Wright, P.V. Reddy, M.P. Deshmukh, R.K. Mathur, M.S. Basu, S. Vasundhara, P. Vindhiya Varman, and A.K. Nagda. 2005. Efficiency of physiological trait–based and empirical selection approaches for drought tolerance in groundnut. Annals Applied Biology 146: 433–439.

Ntare, B. R., and J. H. Williams. 1998a. Heritability and genotype x environment interaction for yield and components of a yield model in segregating population of groundnut under semi-arid conditions. African Crop Science Journal 6(2): 119 - 127.

Ntare, B.R., and J.H. Williams. 1998b. Heritability of component of a simple physiological model for yield in groundnut under semi–arid rainfed condition. Field Crop Research 58: 25–33.

Ogbonnaya, C.I., B. Sarr, C. Brou, O. Diouf, N.N. Diop, and H. Roy-Macauley. 2003. Selection of cowpea genotypes in hydroponics, pots, and field for drought tolerance. Crop Science 43:1114-1120.

Oupadissakoon, C., C.T. Young, and R.W. Mozingo. 1980. Evaluation of free amino acids and free sugars in five lines of Virginia–type peanuts at four locations. Journal of Agricultural and Food Chemistry 29: 800–802.

Ozias-Akins, P. and R. Gill. 2001. Progress in the development of tissue culture and transformation methods applicable to the production of transgenic peanut. Peanut Science 28:123-131.

Pallas, J.E., J.R. Stansell, and T.J. Koske. 1979. Effect of drought on Florunner peanuts. Agronomy Journal 24: 355–359.

Pallas, J.E., Jr., J.R. Stansell, and R.R. Bruce. 1977. Peanut seed germination as related to soil water regime during pod development. Agronomy Journal 69: 381-383.

Pandey, R.K., and J.W. Pendleton. 1986. Genotypic variation in root and shoot growth of peanut in hydroponics. Philippine Journal of Crop Science 11: 189-193.

Pandey, P.K., W.A.T. Herrera, A.N. Villegas, and J.W. Pendleton. 1984. Drought response of grain legumes under irrigation gradient III. Plant growth. Agronomy Journal 76: 557–560.

Passi, S., M. Nazzaro–Porro, C. Fanelli, A.A. Fabbri, and P. Fasella. 1984. Role of lipoperoxidation in aflatoxin production. Applied Microbiology and Biotechnology 19:186–190.

Passioura, J. B. 1972. The effect of root geometry on the yield of wheat growing on stored water. Australian Journal of Agricultural Research 23: 745-752.

Passioura, J. B. 1982. The role of root system characteristics in the drought resistance of crop plant. pp 71-82. *In*: Drought Resistance in Crops With emphasis on Rice. IRRI, Los Baños, Philippines.

Passioura, J. B. 1983. Roots and drought resistance. Agricultural Water Management 7: 265–280.

Passioura, J. B. 1986. Resistance to drought and salinity: Avenues for improvement. Australian Journal of Plant Physiology 13: 191 - 201.

Pattee, H.E., T.G. Isleib, F.G. Giesbrecht, and R.F. McFeeters. 2000. Investigations into genotypic variations of peanut carbohydrates. Journal of Agricultural and Food Chemistry 48: 750–756.

Payne, G.A., D.L. Thompson, E.B. Lillehoj, M.S. Zuber, and C.R. Adkins. 1988. Effect of temperature on the preharvest infection of maize kernels by *Aspergillus flavus*. Phytopathology 78: 1376–1380.

Porter, D.M., and J.C. Smith. 1974. Fungal colonisation of peanut fruit as related to southern corn rootworm injury. Phytopathology 64: 249–251.

Price, A.H., A.D. Tomos, and D.S. Virk. 1997. Genetic dissection of root growth in rice (*Oryza sativa* L.) I; a hydroponic screen. Theoretical and Applied Genetics 95: 132-142.

Puntase J., C. Senthong, S. Meechoui and K.T. Ingram. 2004. Effect of root exudates on drought and aflatoxin resistance of peanut genotypes. *In* Proceedings of the 4th International Crop Science Congress Brisbane, Australia, 26 September – 1 October 2004 (cited 20 Aug 2008). Available from: URL: www.cropscience.org.au .

Rao, K. S., and P. G. Tulpule. 1967. Varietal differences of groundnut in the production of aflatoxin. Nature 214:738-39.

Rao, R.C.N., S. Singh, M.V.K. Sivakumar, K.L. Srivastava, and J.H. Williams. 1985. Effect of water deficit at different growth phases of peanut. I. Yield responses. Agronomy Journal 77: 782-786.

Ravindra, V., P.C. Nautiyal, and Y.G. Joshi. 1990. Physiological analysis of drought resistance and yield in groundnut (*Arachis hypogaea* L). Tropical Agriculture, Trinidad 67: 290-296.

Reddy, T.Y., V.R. Reddy, and V. Anbumozhi. 2003. Physiological responses of peanut (*Arachis hypogaea* L.) to drought stress and its amelioration: A critical review. Plant Growth Regulator 41: 75–88.

Richardson A.D., S.P. Duigan and G.P. Berlyn. 2002. An evaluation of noninvasive methods to estimate foliar chlorophyll content. New Phytologist 153: 185–194.

Ritchie, S.W., H.T. Nguyen, and A.S. Holaday. 1990. Leaf water content and gas exchange parameters of two wheat genotypes differing in drought resistance. Crop Science 30: 105–111.

Robertson, W.K., L.C. Hammond, J.T. Johnson, and K.J. Boote. 1980. Effects of plant–water stress on root distribution of corn, soybeans, and peanuts in sandy soil. Agronomy Journal 72: 548–550.

Rucker, K.S., C.K. Kvien, C.C. Holbrook, and J.E. Hook. 1995. Identification of peanut genotypes with improved drought avoidance traits. Peanut Science 22: 14–18.

Sanders, T.H., R.J. Cole, P.D. Blankenship, and J.W. Dorner. 1993. Aflatoxin contamination of peanuts from plants drought stressed in pod or root zones. Peanut Science 20: 5–8.

Sanders, T.H., R.J. Cole, P.D. Blankenship, and R.A. Hill. 1985. Relation of environmental stress duration to *Aspergillus flavus* invasion and aflatoxin production in preharvest peanuts. Peanut Science 12: 90–93.

Santarius, K. A. 1967. Assimilation of CO2, NADP and PGA reduction and ATP synthesis in intact leaf cells in relation to water content. Planta 73: 228-242.

Sargeant, K. A. Sheridan, J. O'Kelly, and R.B.A Carnaghan. 1961. Toxicity associated with certain samples of groundnuts. Nature 192: 1096-1097.

SAS Institute. 1990. SAS/STAT 6.12; SAS/STAT User's Guide. SAS Institute, Cary, North Carolina.

SAS Institute Inc. 2003. Version 9.1. SAS Inst. Inc., Cary, North Carolina.

Savage, G.P., and J. Keenan. 1994. Composition and nutritive value of peanut kernels, pp. 173–213. *In* J. Smartt (ed.), The groundnut crop. Chapman and Hall: London.

Saxton, A.M. 1998. A macro for converting mean separation output to letter groupings in PROC MIXED, pp. 1243–1246. *In* Proceeding of 23rd Annual SAS Users Group Int. Conf. SAS Inst., Cary, North Carolina, USA.

Shapira, R., N. Paster, O. Eyal, M. Menasherov, A. Mett, and R. Salomon. 1996. Detection of aflatoxigenic molds in grains by PCR. Applied and Environmental Microbiology 62: 3270–3273.

Sheshshayee, M.S., H. Bindumadhava, N.R. Rachaputi, T.G. Prasad, M. Udayakumar, G.C. Wright, and S.N. Nigam. 2006. Leaf chlorophyll concentration relates to transpiration efficiency in peanut. Annals of Applied Biology 148: 7–15.

Serraj, R. 2008. Screening and trait-based selection for drought resistance in rice. (cited on September 1, 2008) Available from: URL: http://www.nrrp.org.la.

Singh, M., R.C.N. Rao, and J.H. Williams. 1991. A statistical assessment of genotypic sensitivity of groundnut (*Arachis hypogaea* L.) to drought in line source sprinkler experiments. Euphytica 57: 19–25.

Singh, M., S. Ceccarelli, and J. Hamblin. 1993. Estimation of heritability from varietal trials data. Theoretical and Applied Genetics 86: 437 - 441.

Singh, S., and M.B. Russel. 1981. Water use by maize/pigeonpea intercrop on a deep Vertisol, Vol. 1, pp. 271–282. *In* Proceedings of International workshop on pigeonpeas. 15–19 December 1980. ICRISAT Center. Patancheru, Andhra Pradesh, India.

Sinclair, T. R. and M. M. Ludlow. 1986. Influence of soil water supply on the plant water balance of four tropical grain legumes. Australian Journal of Plant Physiology 13: 329 – 341.

Singleton, J.A., D.T. Grimm, and T.H. Sanders. 1996. Interference of amino acids in pulsed amperometric detection of peanut sugars. Peanut Science 23: 61–65.

Sivakumar, M.V.K., N. Seetharama, K.S. Gill, and R.C. Sachan. 1981. Response of sorghum to moisture stress using line source sprinkler irrigation. 1. Plant-water relations. Agricultural Water Management 3: 279-289.

Sobolev, V., B. Guo, C.C., Holbrook, and R.E. Lynch. 2007. Interrelationship of phytoalexin production and disease resistance in selected peanut genotypes. Journal of Agricultural and Food Chemistry 55: 2195-2200.

Songsri, P., S. Jogloy, C.C. Holbrook, T. Kesmala, N. Vorasoot, C. Akkasaeng, and A. Patanothai. 2008a. Association of root, specific leaf area and SPAD chlorophyll meter reading to water use efficiency of peanut under different available soil water. Agricultural Water Management 96: 790-798.

Songsri, P., S. Jogloy, N. Vorasoot, C. Akkasaeng, A. Patanothai, and C.C. Holbrook. 2008b. Root distribution of drought-resistant peanut genotypes in response to drought. Journal of Agronomy and Crop Science 194: 92–103.

Songsri, P., S. Jogloy, T. Kesmala, N. Vorasoot, C. Akkasaeng, A. Patanothai, and C. C. Holbrook. 2008c. Heritability of drought resistance traits and correlation of drought resistance and agronomic traits in peanut. Crop Science 48, 2245 – 2253.

Sorensen, V.M., R.J. Hanks, and R.L. Cartee. 1980. Cultivation during early season and irrigation influences on corn production. Agronomy Journal 72: 266-270.

Sorenson, W.G., C.W. Hesselti, and O.L. Shotwell. 1967. Effect of temperature on production of aflatoxin on rice by *Aspergillus flavus*. Mycopathologia et Mycologia Applicata 33: 49.

Squire, R.A.. 1989. Ranking animal carcinogens: a proposed regulatory approach. Science. 214: 887–891.

Stalker, H. T. 1992. Utilization Arachis germplasm resources, pp. 281-295. Groundnut-A Global Perspective. *In* Proceeding of International Workshop S. N. Nigam (ed.). 25-29 Nov. 1991. ICRISAT, Andhra Pradesh, India.

Stanciel, K., D.G. Mortley, D.R. Hileman, P.A. Loretan, C.K. Bonsi, and W.A. Hill. 2000. Growth, pod, and seed yield, and gas exchange of hydroponically grown peanut in response to CO_2 enrichment. Horticultural Science 35: 49-52.

Subbarao, G. V., C. Johansen, A. E. Slinkard, R. C. Nageswara Rao, N. P. Saxena, and Y. S. Chauhan. 1995. Strategies for improving drought resistance in grain legumes. Critical Reviews in Plant Sciences 14: 469 - 523.

Subramanyam, P., and A.S. Rao. 1977. Fungal infection of groundnut pods and aflatoxin accumulation before harvest, pp. 432–443. *In* Proceedings of the Indian Academy of Sciences Section B 85.

Surihan, B., A. Patanothai, and S. Jogloy. 2005. Gene effect for specific leaf area and harvest index in peanut (*Arachis hypogaea* L.). Asian Journal of Plant Science 4(6): 667–672.

Taiz, L., and E. Zeiger. 2006. Stress physiology, pp. 671–681. *In* L. Taiz, and E. Zeiger (eds.), Plant physiology 4th edition. Sinauer Associates, Inc.: Massachusetts.

Thompson, D.L., E.B. Lillehoj, K.J. Leonard, W.F. Kwolek, and M.S. Zuber. 1980. Aflatoxin concentration in corn as influenced by kernel development stage and post inoculation temperature in controlled environments. Crop Science 20: 609–612.

Timper, P., C.C. Holbrook, and D.M. Wilson. 2007. Root vs pod infection by root–knot nematodes on aflatoxin contamination of peanut. Communications in Agricultural and Applied Biological Sciences 72(3): 655–658.

Timper, P., D.M. Wilson, C.C. Holbrook, and B.W. Maw. 2004. Relationship between Meloidogyne arenaria and aflatoxin contamination on peanut. Journal of Nematology 36: 167–170.

Tripathy, J.N., J. Zhang, S. Robin, and H.T. Nguyen. 2000. QTLs for cell–membrane stability mapped in rice (*Oryza sativa* L.) under drought stress. Theoretical and Applied Genetics 100: 1197–1202.

Tulpule, P. G., Bhat, R. V., Nagraj, V. 1977. Variations in aflatoxin in production due to fungal isolates and crop genotypes and their scope in prevention of aflatoxin production. Arch. Inst. Pasteur Tunis 54:487-93.

Turk, K.J., A.E. Hall, and C.W. Asbell. 1980. Drought adaptation of cowpea. I. Influence on corn production. Agronomy Journal 72: 413-420.

Turner, N. C. 1986a. Adaptation to water deficits: A changing perspective. Australian Journal of Plant Physiology 13: 175–190.

Turner, N. C. 1986b. Crop water deficits: A decade of progress. Advance in Agronomy 39: 1–51.

Turner, P. C., M. Mendy, H. White, M. Fortuin, A. J. Hall, and C. P. Wild. 2000. Hepatitis B infection and aflatoxin biomarker levels in Gambian children. Tropical Medicine and International Health 5: 837–841.

Upadhyaya, H. D. 2005. Variability for drought resistance related traits in the mini core collection of peanut. Crop Science 45: 1432–1440.

Utomo, S.D., W.F. Anderson, J.C. Wynne, M.K. Beute, W.M. Jr. Hagler, and G.A. Payne. 1990. Estimates of heritability and correlation among three mechanisms of resistance to Aspergillus parasiticus in peanut. Vol. 22 pp. 26. *In* Proceedings of the American Peanut Research and Education Society. Stone Mountain, Georgia, USA.

Vorasoot, N., C. Akkasaeng, P. Songsri, S. Jogloy, and A. Patanothai. 2004. Effect of available soil water on leaf development and dry matter partitioning in 4 cultivars of peanut (*Arachis hypogaea* L.). Songklanakarin Journal Science and Technology 26(6): 787–794.

Vorasoot, N., P. Songsri, C. Akkasaeng, S. Jogloy, and A. Patanothai. 2003. Effect of water stress on yield and agronomic characters of peanut (*Arachis hypogaea* L.). Songklanakarin Journal Science and Technology 25(3): 283–288.

Waliyar, F., A. Ba, H. Hassan, S. Bonkoungou, and J.P. Bosc. 1994. Sources of resistance to *Aspergillus flavus* and aflatoxin contamination in groundnut genotypes in West–Africa. Plant Disease 78: 704–708.

Wallace, D. H ., J. P. Baudoin, J. Beaver, D. P. Coyne, D. E. Halseth, P. N. Masaya, H. M. Munger, J. R. Myers, M. Silberrnagel, K. S. Yourstone, and R. W. Zobel. 1993. Improving efficiency of breeding for higher crop yield. Theoretical and Applied Genetics 86: 27 - 40.

Wiebe, H. H. 1980. Morphological adaptations to water stress. pp 439-443, *In* Turner, N. C. and P.J. Kramer (eds), Adaptation of Plant to Water and High Temperature Stress. Wiley. New York.

Wild, C.P., and A. J. Hall. 2000. Primary prevention of hepatocellular carcinoma in developing countries. Mutation Research 462: 381–383.

Williams, J.H., and K.J. Boote. 1995. Physiology and modeling – predicting the unpredictable legume, pp. 302–353. *In* H.E. Patte, and H.T. Stalker (eds.), Advanced in legume science. American Peanut Research Association: Oklahoma. USA.

Wilson, D.M., and J.R. Stansell. 1983. Effect of irrigation regimes on aflatoxin contamination of peanut pods. Peanut Science 10: 54–56.

Wotton, H. R., and R.N. Strange. 1985. Circumstantial evidence for phytoalexin involvement in the resistance of peanuts to Aspergillus flavus. Journal of General Microbiology 131: 487-494.

Wotton, R., and R.N. Strange. 1987. Increased susceptibility and reduced phytoalexin accumulation in drought stressed peanut kernels challenged with *Aspergillus flavus*. Applied and Environmental Microbiology 53: 270–273.

Wright, G. C., and G. L. Hammer. 1994. Distribution of nitrogen and radiation use efficiency in peanut canopies. Australian Journal of Agricultural Research 45: 565–574.

Wright, G.C., and R.B. Hansen. 1997. Climatic effects on aflatoxin incidence and management in peanut, pp. 62–65. *In* Proceedings of 2nd Australian peanut conference. Department of Primary industries. Gold Coast, Queensland, Australia.

Wright, G.C., and R.C. Nageswara Rao. 1994a. Groundnut water relations, pp. 281–325. *In* J. Smartt (ed.), The groundnut crop. A Scientific Basis for Improvement. Chapman and Hall: London.

Wright, G.C., and R.C. Nageswara Rao. 1994b. Selection for water-use efficiency in grain legumes, pp. 70. *In* G.C. Wright, and R.C. Nageswara Rao (eds.), Report of a workshop. ACIAR Technical reports No.27. 5-7 May 1993. ICRISAT Center, Andhra Pradesh, India,

Wright, G. C., K. T. Hubick, and G. D. Farquhar. 1988. Discrimination in carbon isotopes of leaves correlates with water-use efficiency of field-grown peanut cultivars. Australian Journal of Plant Physiology 15: 815 - 825.

Wright, G. C., K.T. Hubick, and G.D. Farquhar. 1991. Physiological analysis of peanut cultivars response to timing and duration of drought stress. Australian Journal Agricultural Research 42: 453–470.

Wright, G.C., R.C. Nagesvara Rao, and G.D. Farquhar. 1994. Water use efficiency and carbon isotope discrimination in peanut under water deficit condition. Crop Science 34: 92–97.

Wright, G.C., R.C. Nageswara Rao, and M.S. Basu. 1996. A physiological approach to the understanding of genotype by environment interactions – A case study on improvement of drought adaptation in groundnut, pp. 365–380. *In* M. Cooper, and G.L. Hammer (eds.), Plant adaptation and crop improvement. CAB International: Wallingford.

Young, C. T. 1980. Amino acid composition of three commercial peanut varieties. Journal of Food Science 45: 1086–1087.

Zharare, G.E., C.J. Asher, F.P.C. Blamey, and P.J.Dart. 1993. Pod development of groundnut (*Arachis hypogaea* L.) in solution culture. Plant and Soil 155/156: 355-358.

Zharare, G.E., F.P.C. Blamey, and C.J. Asher. 1998. Initiation and morphogenesis of groundnut (*Arachis hypogaea* L.) pods in solution culture. Annals of Botany 81: 391-396.

Zuber, M.S., L.L. Darrah, E.B. Lillehoj, L.M. Josephson, A. Manwiller, G.E. Scott, R.T. Gudauskas, E.S. Horner, N.W. Widstrom, D.L. Thompson, A.J. Bockholt, and J.L. Brewbaker. 1983. Comparison of open pollinated maize varieties and hybrids for preharvest aflatoxin contamination in the south United States. Plant Disease 67: 185–187.