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Heritability of N_2 Fixation Traits, and Phenotypic and Genotypic Correlations between N_2 Fixation Traits with Drought Resistance Traits and Yield in Peanut

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ABSTRACT

Drought stress reduces growth and yield in peanut (Arachis hypogaea L.) and also reduces nitrogen fixation (NF). Peanut production in drought prone areas should be enhanced by the development of cultivars that can fix more nitrogen (N) under drought conditions. The aims of this study were to estimate heritability for NF and to estimate phenotypic and genotypic correlations among traits related to NF with drought-resistance traits and yield under well-watered and drought conditions. A total of 140 lines in the $F_{4:7}$ and $F_{4:8}$ generations derived from four crosses, parental lines, and a non-nodulating line as a non-N_2-fixing reference plant were evaluated in the dry seasons 2005/2006 and 2006/2007. These lines were evaluated in rhizobium inoculated soil without N fertilizer under field capacity (FC) and 2/3 available soil water (AW). Data were recorded for specific leaf area (SLA), SPAD chlorophyll meter reading (SCMR), nodule number (NN), nodule dry weight (NDW), shoot dry weight (SDW), pod dry weight (PDW), total dry weight (TDW), harvest index (HI), and NF. Broad-sense heritability (h^2_b) and narrow-sense heritability (h^2_n) estimates for NF under FC and 2/3 AW were in the same ranges (h^2_b = 0.84 to 0.98 and h^2_n = 0.29 to 0.39). Positive relationships between NF under FC and 2/3 AW (r = 0.73, $P \leq 0.01$) indicate that selection for the lines that fixed high N under well-watered conditions should produce lines that fixed high N under drought conditions. Selection for NF under drought conditions might be more effective in improving yield because of a higher correlation between NF and PDW (r_G = 0.43, $P \leq 0.01$) under drought conditions than under FC (r_G = 0.13). The use of SCMR and SLA as surrogate traits for NF would be less effective than direct selection because of weak correlations between these traits and NF.

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Abbreviations: AW, available soil water; DAP, days after planting; DSC, drought stress conditions; FC, field capacity; HI, harvest index; N, nitrogen; NA, nitrogenase activity; NF, nitrogen fixation; NN, nodule number; NDW, nodule dry weight; PDW, pod dry weight; R, reduction; SDW, shoot dry weight; Es, surface evaporation; SLA, specific leaf area; SCMR, SPAD chlorophyll meter reading; TDW, total dry weight.

Peanut (*Arachis hypogaea* L.) is grown mainly in semiarid tropic regions, which are characterized by low soil fertility and unpredictable rainfall. The limitation in available soil N causes low productivity of several crops in these areas. Atmospheric N can be assimilated into a useful form through symbiotic nitrogen fixation (NF) by legumes, including peanut, in association with specific *Rhizobium* or *Bradyrhizobium*. As N is an essential nutrient for growth and yield of peanut, especially in infertile soil and under drought conditions, high NF genotypes would be expected to give higher yield under drought. Much research has been conducted to improve selection efficiency in peanut by exploring NF and its surrogate traits for improving yield (Bado et al., 2006; Pimratch et al., 2004a, 2004b).

Nitrogen is related to chlorophyll content that partially determines photosynthetic capacity of plants. More recently, Bado et al. (2006) clearly demonstrated a significant correlation between total N yield and N fixed, indicating a significant contribution of

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fixed N to peanut growth. Pimratch et al. (2008) reported that the ability to maintain high NF under drought stress could aid peanut genotypes in maintaining high yield under water-limited conditions. However, selection for high yield under drought conditions has been the main strategy for drought-resistance breeding of peanut. This procedure has made only slow progress because of the complex nature of the trait. More effective strategies are needed. Simple traits, such as specific leaf area (SLA) and SPAD chlorophyll meter reading (SCMR), have been proposed as surrogate traits for drought tolerance (Wright et al., 1994; Nageswara Rao et al., 2001; Sheshshayee et al., 2006; Upadhyaya, 2005; Lal et al., 2006).

The relationships between yield and NF and its surrogate traits as mentioned above led us to investigate whether these traits could be used effectively in breeding peanut for improved yield under drought conditions. For practical breeding, information on the inheritance of the traits is important. Arrendell et al. (1985) found that broad-sense heritability estimates for nodule number (NN), nodule dry weight (NDW), nitrogenase activity (NA), shoot dry weight (SDW), and pod dry weight (PDW) were moderate to high. Provorov and Tikhonovich (2003) reported high heritability of nitrogenase activity (NA) in many leguminous crops, suggesting that selection for this activity may be effective. Pimratch et al. (2004b) found that most of the heritability estimates for NF in the F_4 generation of six peanut crosses were moderate to high (0.22 to 0.88) and the values of heritability estimates varied depending on crosses. Heritability estimates for NF were relatively high (0.84 to 0.98) in the F_5 generation (Sikinarum et al., 2006). Most reports on genetic studies for NF and related traits in peanut have been investigated under well-water conditions, and information under drought conditions is lacking. Drought might alter the performance of peanut genotypes and the information under well-watered conditions may not be applicable to drought conditions. Therefore, investigations under drought conditions are needed.

Selection for drought resistance using pod yield under drought conditions as the selection criterion has produced only slow progress due to large genotype x environment interactions (Wright et al., 1994; Nageswara Rao and Wright, 1994; Sheshshayee et al., 2006). The integration of physiological traits (or their surrogates) in the selection scheme would be advantageous in selecting genotypes that are more efficient water utilizers or partitioners of photosynthates into economic yield (Nigam et al., 2005). Using surrogate traits for drought resistance can lead to more rapid progress because of their simple inheritance. The increase of NF under drought stress conditions may also be possible through selection for high SCMR, low SLA, high harvest index (HI), and acceptable yield if NF is genetically associated with one or more of these traits. Selection for NF might affect pod yield and surrogate traits for drought resistance, and, alternatively, it might be possible to use drought resistance traits to improve NF.

Development of peanut varieties with high NF and high yield under drought conditions is the main objective of the ongoing peanut breeding program at Khon Kaen University. Up to the present time, most research aiming to improve NF in peanut crop, including our work (Pimratch et al., 2004a, 2004b; Sikinarum et al., 2006), has been conducted under well-watered conditions. Cultivars developed through this process may not perform well under drought. The heritability and selection efficiency under drought and well-watered conditions might be different and optimum conditions for effective selection have to be identified.

The objectives of this study were: (i) to estimate broad- and narrow-sense heritability for NF under well-watered conditions and drought stress conditions and (ii) to estimate phenotypic and genotypic correlations among traits related to NF and SLA, SCMR, pod yield, and HI under well-watered and drought conditions.

MATERIALS AND METHODS

Plant Materials

Two drought resistant lines (ICGV 98308 and ICGV 98324; 110-d maturity) of peanut received from the International Crops Research Institute for Semi-Arid Tropics (ICRISAT) (Nigam et al., 2003; 2005) were used as female parents and crossed with two well-adapted and high-yielding cultivars KK 60-3 (120-d maturity) and Tainan 9 (100-d maturity). The drought resistant lines are Spanish-type peanut with moderate and low NF, respectively (Pimratch et al., 2008). KK 60-3 is a Virginia-type peanut cultivar with high NF (Toomsan et al., 1995; Pimratch et al., 2008), but sensitive to drought for pod yield, and Tainan 9 is a Spanish-type peanut cultivar having low dry-matter production (Vorasoot et al., 2003) and low NF (McDonagh et al., 1993; Pimratch et al., 2008). The resulting four F_1 hybrids (ICGV 98308 × KK 60-3, ICGV 98308 × Tainan 9, ICGV 98324 × KK 60-3, and ICGV 98324 × Tainan 9) were allowed to self-pollinate to produce ample F_2 seeds. In F_2 and F_3 generations, two pods were kept for each plant and bulked in each cross. Line separation was performed in the F_4 generation. A total of 140 lines (35 lines for each cross) were randomly selected and multiplied in the F_5 and F_6 generations.

Experimental Procedures

The experiment was conducted for two years under field conditions at the Field Crop Research Station of Khon Kaen University located in Khon Kaen province, Thailand (latitude 16° 28′ N, longitude 102° 48′ E, 200 m above sea level) during October 2005 to April 2006 (dry season 2005/2006), and repeated during October 2006 to April 2007 (dry season 2006/2007), using $F_{4:7}$ and $F_{4:8}$ lines, respectively. The soil type is Yasothon series (Yt; fine-loamy, siliceous, isohypothermic, Oxic Paleustults) with pH 6.23, 0.848% organic matter, and 0.035% total N (based on analysis of samples from a depth of 0–15 cm). The proportions of sand, silt, and clay in the soil were 93.93, 3.57, and 2.50%, respectively. Available P was 62.02 mg kg^{-1} and extractable K and Ca were 64.40 and 543.45 mg kg^{-1}, respectively.

Before initiation of experiments, soils were analyzed by the pressure plate method to determine water holding capacity of the soils. At FC, the soil water holding capacity was 11.0%, and permanent wilting point was 4.6%. Therefore, soil water holding capacities at 2/3 AW was determined at 8.8%.

The four parental genotypes were included for comparison purposes and a non-nodulating line (McDonagh et al., 1993) was also included as reference plant for N determination. A total of 145 entries were tested in a split-plot design with four replications. Two water regimes FC (11.0%) and 2/3 AW (8.8%) in 0 to 60 cm depth were assigned as main plots and peanut genotypes as subplots. Each entry was planted in five row plots 3.2 m long. Spacing was 50 cm between rows and 20 cm between hills within the row.

Soil preparation was done by plowing the field three times. Lime at the rate of 625 kg ha^{-1}, phosphorus fertilizer as triple superphosphate at the rate of 24.7 kg P ha^{-1}, and potassium fertilizer as muriate of potash (KCl) at the rate of 31.1 kg K ha^{-1} were applied before 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} seed before planting. The seeds were treated with ethrel 48% at the rate of 2 mL L^{-1} water, to break possible dormancy and to ensure uniform germination. Plots were over-planted and the seedlings were later thinned to two plants per hill at 21 d after planting (DAP). Rhizobium inoculation was applied by spraying a water-diluted commercial peat-based inoculum of *Bradyrhizobium* (mixture of strains THA 201 and THA 205; Dep. of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand) on the rows. Weeds were controlled by an 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 hoeing during the growing 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-dimethyl-benzofuran-7-yl (dibutylaminothio) methylcarbamate 20% (w/v) water soluble concentrate] at 2.5 L ha^{-1}, methomyl {*S*-methyl-*N*-[(methylcarbamoyl)oxy] thioacetimidate 40% soluble powder} at 1.0 kg ha^{-1}, and carboxin [5,6-dihydro-2-methyl-1,4-oxathine-3-carboxanilide 75% wettable powder] at 1.68 kg ha^{-1}.

Subsurface drip-irrigation was installed to irrigate the crop, and soil water level was maintained at FC (102.63 mm in 60 cm depth) from sowing until 21 DAP in all treatments to support crop establishment. Afterward, soil moisture for the stress treatment plots were allowed to gradually decline until reaching the predetermined level of 2/3 AW at 0 to 60 cm (82.57 mm) at 28 DAP, and then were held more or less constant until harvest. In maintaining the specified soil moisture levels, water was added to the respective plots by subsurface drip-irrigation on the basis of crop water requirements and surface evaporation, which were calculated following the methods described by Doorenbos and Pruitt (1992) and Singh and Russell (1980), respectively.

Total crop water use for each water treatment was calculated as the sum of transpiration and soil evaporation. Transpiration was calculated using the methods described by Doorenbos and Pruitt (1992):

$$ETcrop = ETo \times Kc$$

where ETcrop = crop water requirement (mm/day), ETo = evapotranspiration of a reference plant under specified conditions calculated by pan evaporation method, Kc = the crop water requirement coefficient for peanut, which varies with genotype and growth stage (Doorenbos and Kassam, 1986).

Surface evaporation (Es) was calculated as (Singh and Russell, 1980):

$$Es = \beta \times (Eo/t),$$

where Es = soil evaporation (mm), β = light transmission coefficient measured depending on crop cover, Eo = evaporation from class A pan (mm/day), t = days from the last irrigation or rain (day).

Soil moistures were measured by the gravimetric method at 0, 37, 52, 67, 82, and 97 DAP and harvest at the depth of 0 to 5, 25 to 30, and 55 to 60 cm. The measurement at planting was for calculating the correct amount of water to be applied to the crop, and the measurement at harvest was for calculating the water use of the crop. Soil moistures were also detected at 7 d intervals using a neutron moisture meter (Type I.H. II SER. No. N0152, Ambe Diccot Instruments Co. Ltd., Abingdon, Oxon, UK) at the depth of 30, 60, and 90 cm to calculate the amount of water to be supplied to the crop.

At 52, 67, 82, and 97 DAP, five plants from each plot were randomly sampled, and the second fully expanded leaf from the tops of the main stem were used for SCMR at 0900 to 1200 h. SPAD chlorophyll meter readings were recorded using a Minolta SPAD-502 m (Tokyo, Japan) on the four leaflets from each leaf. The detailed sampling procedures have been described elsewhere (Nageswara Rao et al., 2001). An average SCMR for each plot was derived from 20 observations. The leaves that were used for SCMR were harvested for SLA. A sample of 20 leaflets was measured with a leaf area meter (LI-COR Area Meter Model 3100, LI-COR Inc., Lincoln, NE, USA). The leaf samples were oven dried at 80°C for at least 48 h and weighed to determine leaf dry weight. The SLA was computed by dividing leaf area with its corresponding dry weight.

For each plot, bordered plants in an area of 4.2 m^2 were harvested at maturity (R8) (Boote, 1982), then depodded, and fresh shoot weight was measured in the field. A random shoot sample of 2 kg for each plot was taken from total shoot fresh weight, oven dried at 80°C for at least 48 h and dry weight was measured. Shoot dry weight of the sample was then converted to shoot weight per plot. Pod dry weight was determined after sun drying of the pods to approximately 8% moisture content. Harvest index was computed by dividing the total pod weight with its corresponding total dry weight (TDW) at the final harvest.

Nodule number and NDW were determined at harvest. Five plants from each plot were randomly chosen and were carefully dug to recover as many nodules as possible. The plants were cut at ground level to separate roots from shoots. The samples were washed with tap water and nodules were removed from roots by hand and counted for NN. The nodules were oven dried at 80°C for 48 h and NDW was measured.

The N-difference method using a non-nodulating line as a reference plant was used for determination of NF because it is a reliable method and economical. This method has been proven in previous studies to be as effective as ^{15}N isotope dilution method in determining N fixation (McDonagh et al., 1993; Bell et al., 1994;

Phoomthaisong et al., 2003). The NF was determined at harvest from shoot excluding pods and roots. The sample was ground using a hammer mill and the N content was analyzed by micro-Kjeidahl digestion (Black, 1965). Total N was then measured using the automated indophenol method (Schuman et al., 1973), and read on a flow injection analyzer model 5012 (Tecator Inc., Hoganas, Sweden). Fixed N contents (shoot) were calculated as follows:

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

Percentage of the reduction in N_2 fixed was calculated as follows:

Percentage of reduction of N_2 fixed = [1 – (N_2 fixed under stress/N_2 fixed under non-stress)] x 100.

Percentages of the reduction of NN, NDW, SDW, PDW, and TDW were calculated in the same manner and were used to evaluate drought sensitivities of peanut genotypes.

Statistical Analysis

Individual analysis of variance was performed for each year using a split-plot design (Gomez and Gomez, 1984). Homogeneity of variance was tested for all characters and combined analysis of variance of 2-yr data was performed. Calculation procedures were done using MSTAT-C package (Bricker, 1989). Because water regimes x genotype interaction was significant, each water regime was analyzed separately according to a randomized complete block design (Gomez and Gomez, 1984) for comparison of heritability, phenotypic, and genotypic correlation between different water regimes.

Broad-sense heritability estimates for each trait of four crosses were calculated on the basis of progeny means for each cross (Holland et al., 2003).

$$h_b^2 = \sigma_G^2/\sigma_P^2$$

where $\sigma^2_P = \sigma^2_G + \sigma^2_{GE}/e + \sigma^2_E/re$, σ^2_G = genetic variances, σ^2_P = phenotypic variances, σ^2_{GE} = genetic x environmental variances, σ^2_E = error variances, e = number of environments, and r = number of replications. The standard errors (SE) of heritability for N_2 fixation traits were calculated to give a measure of the precision of the estimates as described by Singh et al. (1993).

Narrow-sense heritability estimates were computed using the regression coefficients of F_8 on F_7 generations means as described by Smith and Kinman (1965).

$$h_n^2 = b_{(F8,F7)}/2r_{op}, \quad h_n^2 = b_{(F8,F7)}/2(127/128)$$

where $b_{(F8,F7)}$ is the regression coefficient of F_8 on F_7 generations and r_{op} is the correlation coefficient of parentage with a value of 127/128 between F_7 and F_8 generations. The standard errors of narrow sense heritability for N_2 fixation traits were calculated using the methods described by Hallauer and Miranda Fo (1988). Heritability estimates were grouped as high (>0.50), moderate (0.20–0.50), and low (<0.20) as suggested by Stansfield (1986).

Phenotypic and genotypic correlation coefficients between nitrogen fixation traits and drought resistance traits were calculated on the basis of progeny means (140 lines) by the method described by Falconer and Mackay (1996).

Phenotypic correlations (r_P) were estimated in the following manner:

$$r_P = M_{12}/[(M_{11})(M_{22})]^{1/2}$$

where M_{12} is the mean cross product for lines and M_{11} and M_{22} are the means squares for lines for characters (numbers 1 and 2) under consideration.

The genotypic correlations (r_G) were estimated in a similar manner:

$$r_G = \mathrm{Cov}_{p12}/(\sigma^2_{p1}\sigma^2_{p2})^{1/2}$$

where Cov_{p12}, σ^2_{p1}, and σ^2_{p2} are estimates of the progeny covariance component between a given pair of characters and the progeny variance components of the characters, respectively. The correlation coefficients with 138 degrees of freedom were tested for significance by comparing calculated values with tabular values. The detailed test method is available in standard statistics books, e.g., Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Rainfall, relative humidity, evaporation, maximum and minimum temperature, and solar radiation were recorded daily from sowing until harvest by a weather station that was 30 m distant from the experimental site. Weather data for both years are presented in Fig. 1. There was maximum rainfall (13.0 mm) at 95 DAP in the dry season 2005/2006 (Fig. 1a), and (39 mm) at 97 DAP in the dry season 2006/2007 (Fig. 1c). Daily pan evaporation ranged from 2.78 to 9.62 mm in 2005/2006 and 2.88 to 9.84 mm in 2006/2007 (Fig. 1a and 1c, respectively). The average humidity in the dry season 2005/2006 (82.5%) was higher than those in the dry season 2006/2007 (76.1%) (Fig. 1a and 1c, respectively). The seasonal mean maximum and minimum air temperature ranged between 32.0°C and 20.0°C in 2005/2006 and 33.0°C and 20.0°C in 2006/2007 (Fig. 1b

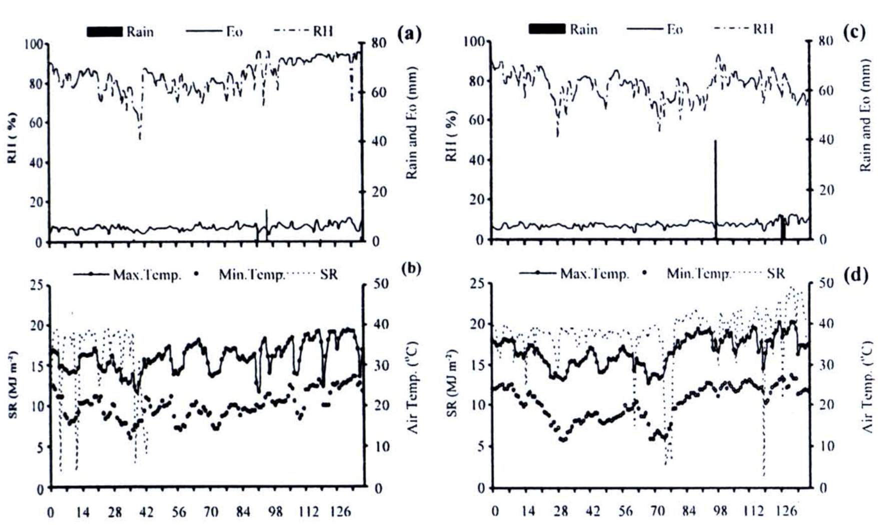

Figure 1. Rain fall, evaporation (E_O), relative humidity (RH), maximum and minimum air temperature (Max and Min Temp.), and solar radiation (SR) in 2005/2006 (a, b) and 2006/2007 (c, d).

and 1d, respectively). The seasonal mean solar radiation was 16.74 MJ m^{-2} d^{-1} in 2005/2006 and 18.76 MJ m^{-2} d^{-1} in 2006/2007 (Fig. 1b and 1d, respectively).

Soil moisture levels for different water regimes, as determined by percentages of available moisture content, at the depths of 0 to 5, 25 to 30, and 55 to 60 cm were different among well-watered and drought conditions for both years (data not reported). Soil moisture volume fractions in the dry seasons 2005/2006 and 2006/2007 were monitored periodically at 30, 60, and 90 cm of soil profiles (Fig. 2). Differences among water regimes were obvious at 30 and 60 cm indicating reasonable control of water treatments. However, at soil depth of 90 cm, the soil moisture volume fractions of all water treatments were not different. Observations found visual wilting in 2/3 AW in the afternoon.

Combined analysis of variance showed significant differences among 140 progenies ($P \le 0.01$) for NF, NN, NDW, SDW, TDW, and the reductions of these traits (data not presented). This indicated that genetic variation existed for these characters, and, thus, estimation of heritability could be performed. Genotypic differences were seen for SLA, SCMR, PDW, and HI for both stress and non-stress conditions. The interaction effects of Y x G were significant ($P \le 0.01$) for NN, NDW, and PDW under well-watered and 2/3 AW conditions. On the basis of a low coefficient of variation and high F-ratio from analysis of variance, the best assessment times for SLA and SCMR was observed at 67 DAP. Thus the evaluation data at 67 DAP are reported.

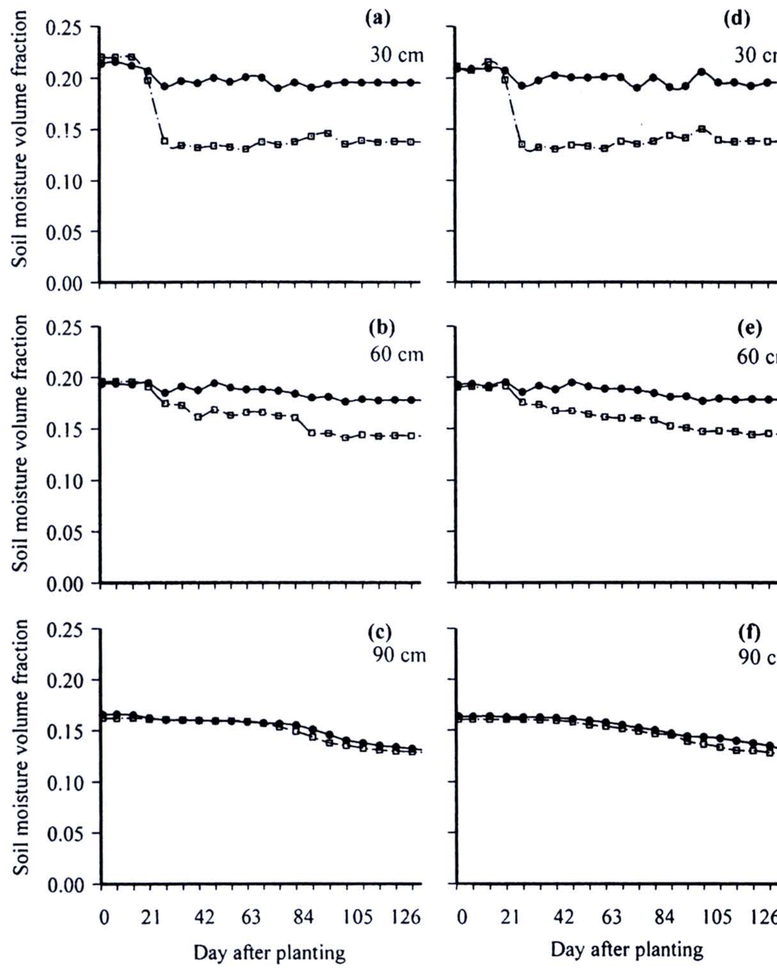

Figure 2. Soil moisture volume fraction in two water regimes [field capacity (FC); ●, and 2/3 available water (AW); □] at the soil depth 30, 60, and 90 cm of dry season 2005/2006 (a, b, and c, respectively) and dry season 2006/2007 (d, e, and f, respectively) estimated by Neutron probe.

Differences in traits related to NF were significant for both well-watered and drought conditions except for NN under well-watered conditions and NDW under drought conditions (Table 1). KK 60-3 had the highest NF, SDW, and TDW under both conditions. The reductions in NN and NDW were statistically significant, whereas the reductions in other characters were not.

The h^2_b estimates for NF in the four crosses were high, ranging from 0.84 ± 0.14 to 0.95 ± 0.06 under well-watered conditions and 0.91 ± 0.09 to 0.93 ± 0.08 under stressed conditions, but the heritability estimates for the reduction in NF were much lower than those for NF, ranging from 0.53 ± 0.27 to 0.79 ± 0.19 (Table 2). Most heritability estimates for NN were moderate and high, ranging from 0.23 ± 0.39 to 0.86 ± 0.13 except for low heritability estimates in the cross ICGV 98324 x KK 60-3 under drought conditions, but most heritability estimates for the reduction in NN were low. The heritability estimates for NDW, SDW, and TDW under well-watered and stressed conditions, ranging from 0.50 ± 0.31 to 0.97 ± 0.04, were generally higher than those for NN. The heritability estimates for the reduction in NDW, SDW, and TDW were lower than those for their actual values, ranging from 0.00 to 0.93 ± 0.11.

Estimates of h^2_n computed by parent offspring regression were generally lower than those computed from variance estimates (Table 2). Narrow-sense heritability estimates for NF in the four crosses were moderate, ranging from 0.29 to 0.39 under well-watered conditions and 0.31 to 0.37 under stressed conditions, and the heritability estimates for the reduction in NF were similar to those for NF, ranging from 0.21 to 0.40 (Table 2). Most heritability estimates for NN were low, ranging from 0.00 to 0.24, except for the rather higher heritability estimate in the cross ICGV 98324 x Tainan 9 (0.40) under well-watered conditions. The heritability estimates for NDW were moderate to high (0.21 to 0.60) under both conditions except for low heritability estimates (0.15 to 0.19) in the cross ICGV 98308 x KK 60-3. The heritability estimates

Table 1. Means for nitrogen fixation (NF), nodule number (NN), nodule dry weight (NDW), shoot dry weight (SDW), total dry weight (TDW), and reduction (R) of these traits of parental lines of peanut evaluated across two environments in dry season 2005/2006 and 2006/2007.

Genotypes	NF			NN			NDW			SDW			TDW		
	FC†	DSC‡	R§	FC	DSC	R	FC	DSC	R	FC	DSC	R	FC	DSC	R
	kg N ha^{-1}		%	nodule plant^{-1}		%	g plant^{-1}		%	tons ha^{-1}		%	tons ha^{-1}		%
ICGV 98308	105.9b¶	92.4b	13.1	321.1	114.0b	62.2a	0.235ab	0.130	42.2ab	5.35b	4.42c	17.7	7.76b	7.13b	7.7
ICGV 98324	98.4b	86.9b	7.9	387.4	172.6ab	54.8a	0.277a	0.140	48.3a	5.10b	4.30c	14.6	7.65b	7.07b	6.6
KK 60-3	171.9a	147.0a	12.9	323.1	154.8ab	52.5a	0.296a	0.134	53.8a	8.28a	6.59a	20.4	10.8a	8.96a	17.1
Tainan 9	101.1b	100.4b	0.8	298.9	207.1a	33.8b	0.181b	0.137	29.8b	5.92b	5.52b	7.4	8.20b	7.74b	5.8
Mean	119.4	108.6	6.9	332.6	162.1	50.9	0.248	0.136	43.6	6.2	5.2	15.1	8.6	7.7	9.4

†FC, field capacity.

‡DSC, drought stress conditions.

§Percentage of reduction (R) = [1 – (trait under stress/trait under non stress)] x 100.

¶Means in the same column followed by the same letter (s) were not statistically significant by DMRT at 0.05 probability level.

for the reduction in NN and NDW were lower than those of their actual values, ranging from 0.00 to 0.11. However, they were lower than those for the reduction in NF. The heritability estimates for SDW and TDW were also moderate under well-watered and stressed conditions when compared to those of NF, ranging from 0.29 to 0.46. The heritability estimates for the reduction in SDW and TDW were similar to those of their actual values, ranging from 0.27 to 0.37 and they were close to those for the reduction in NF.

Broad-sense heritability estimates in general were much higher than h^2_n estimates (Table 2). Similar results were reported by Chiow and Wynne (1983), who found that h^2_b estimates using variance components were higher than h^2_n estimates using the parent offspring regression. As the evaluation of heritability estimates was conducted in late generations (F_7 and F_8) 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 generational advance (Holland, 2001). The moderate and high heritability estimates for NF and its related traits under well-watered and drought conditions indicated that the improvement for these individual traits would be possible in this population and the selection under drought or well-watered conditions can be practiced with similar progress.

The low heritability estimates for NN may be due to a lack of genetic variation among the parents that we used (Table 1). Similar results of relatively low h^2_b estimates for NN have been observed in other studies (Pimratch et al., 2004b; Phudenpa et al., 2005). Arrendell et al. (1988) also reported very low realized heritability estimates for NN (0.05) and NDW (0.06). Cruickshank et al. (2004) reported that the heritability estimates varied significantly between crosses and traits depending on levels of genetic variation in parents. In some cases the variation seems to be caused by errors in sampling method. This might also suggest the high environmental variation for nodule traits because of difficulty in recovery of nodules from soils and the decay of nodules at harvest.

Results from selection for NF and related traits in peanut under well-watered conditions have been reported (Arrendell et al., 1985; Pimratch et al., 2004b; Bado et al., 2006; Sikinarum et al., 2006). The selection for these traits under drought condition was the aim in this study. If selection for genotypes with high NF under well-watered conditions is similar to selection under drought conditions, then information on selection for NF under well-watered conditions may be applicable to drought conditions. The correlation coefficient between NF under well-watered and under drought conditions of peanut lines was high ($r = 0.77$, $P \leq 0.01$) (Table 2) and the correlation coefficients for NN, NDW, SDW, and TDW were similar, ranging from 0.67, to 0.81 ($P \leq 0.01$). Positive and significant correlation coefficients between well-watered and drought conditions for NF and its related traits suggested that selection for lines that fixed high N under well-watered conditions should produce lines that have high NF under drought conditions, and the decision of breeders can be based on the degrees of association and cost of operation.

Selection for NF, SDW, and TDW would be more effective than those for NN and NDW under both stress and non-stress conditions because of their higher heritability estimates. Selection for NF and its related traits under non-stressed conditions and stressed conditions should be equally effective and yield similar results. In terms of simplicity and effective use of available resources, selection under well-watered conditions is recommended.

We examined the correlations of NF and its related traits with PDW, HI, SCMR, and SLA to better understand these relationships and to assess whether SCMR and SLA could be used as selection tools to enhance NF. Data of the experiment under well-watered and drought conditions were compared to identify optimum conditions for selection. The drought-induced reductions in NF and its related traits were also correlated with the reductions in pod yield (Table 3). The values for r_G and r_P were similar and, thus, this discussion is focused on r_G.

Under well-watered conditions, NF and SDW were not correlated with PDW but NF and SDW were negatively correlated with HI (r_G = −0.46 to −0.48, $P \leq 0.01$) (Table 3). The results indicated that NF under well-watered conditions contributed to vegetative growth rather than to yield. However, these traits were positively correlated with SCMR (r_G = 0.20 to 0.23, $P \leq 0.01$) while NN and NDW, were significantly correlated with SLA and PDW. NN and NDW were significantly correlated with SLA but other traits were not. This might suggest that leaf area is necessary for maintaining photosynthesis that provides carbon for nodule formation and maintenance. However, leafy plants may not produce high pod yield. TDW was significantly correlated with SCMR (r_G = 0.41, $P \leq 0.01$) and PDW (r_G = 0.61, $P \leq 0.01$). In a related study previously reported by Songsri et al. (2008), SCMR was positively correlated with pod yield under both drought-stressed (r_G = 0.21, $P \leq 0.05$) and well-watered (r_G = 0.51, $P \leq 0.01$) conditions. Their results suggested that these traits were associated, especially under well-watered conditions, suggesting that it might be possible to use SCMR as a surrogate trait for NF. Because crops with higher biomass production require more N, biomass production was closely correlated with NF (Pimratch et al., 2004a; Herridge and Rose, 2000). However, the contribution of biomass production to pod yield was dependent partly on HI (Songsri et al., 2008). The correlation coefficients between TDW and PDW were higher under well-irrigated conditions. This also suggested that, under well-watered conditions, pod yield is largely dependent on biomass production. However, biomass production was dependent on chlorophyll content in leaves which could be measured indirectly by a handheld portable SPAD chlorophyll meter (Samdur et al., 2000; Nageswara Rao et al., 2001; Akkasaeng et al., 2003).

Under drought conditions, positive and significant phenotypic and genotypic correlations were observed between NF, NN, NDW, SDW, and TDW and PDW (r_G = 0.43 to 0.78, $P \leq 0.01$) (Table 3). The results indicated that pod yield under drought was more dependent on NF

Table 2. Broad sense heritability and narrow sense heritability estimates for nitrogen fixation (NF), nodule number (NN), nodule dry weight (NDW), shoot dry weight (SDW), total dry weight (TDW), and the reductions in these traits under well-watered and drought stress conditions of four crosses of peanut evaluated across two environments in dry season 2005/2006 and 2006/2007.

Peanut crosses	Traits related to nitrogen fixation									
	Broad-sense heritability					Narrow-sense heritability				
	NF	NN	NDW	SDW	TDW	NF	NN	NDW	SDW	TDW
Well-watered conditions										
ICGV 98308 x KK 60-3	0.84 ± 0.14‡	0.23 ± 0.39	0.55 ± 0.29	0.91 ± 0.10	0.89 ± 0.12	0.29 ± 0.03	0.10 ± 0.06	0.19 ± 0.04	0.37 ± 0.02	0.37 ± 0.02
ICGV 98308 x Tainan 9	0.95 ± 0.06	0.39 ± 0.34	0.69 ± 0.22	0.97 ± 0.04	0.98 ± 0.03	0.36 ± 0.05	0.13 ± 0.07	0.38 ± 0.06	0.43 ± 0.04	0.45 ± 0.03
ICGV 98324 x KK 60-3	0.86 ± 0.11	0.40 ± 0.34	0.64 ± 0.25	0.92 ± 0.08	0.93 ± 0.08	0.36 ± 0.04	0.18 ± 0.05	0.21 ± 0.05	0.40 ± 0.03	0.41 ± 0.03
ICGV 98324 x Tainan 9	0.92 ± 0.08	0.86 ± 0.13	0.86 ± 0.13	0.95 ± 0.06	0.98 ± 0.03	0.39 ± 0.04	0.40 ± 0.04	0.54 ± 0.05	0.42 ± 0.03	0.46 ± 0.03
Drought conditions										
ICGV 98308 x KK 60-3	0.91 ± 0.09	0.30 ± 0.37	0.50 ± 0.31	0.94 ± 0.07	0.94 ± 0.07	0.31 ± 0.05	0.13 ± 0.07	0.15 ± 0.07	0.35 ± 0.03	0.36 ± 0.03
ICGV 98308 x Tainan 9	0.91 ± 0.09	0.59 ± 0.27	0.75 ± 0.19	0.86 ± 0.13	0.93 ± 0.07	0.31 ± 0.05	0.24 ± 0.07	0.55 ± 0.09	0.29 ± 0.03	0.34 ± 0.03
ICGV 98324 x KK 60-3	0.93 ± 0.08	0.00 ± 0.00	0.50 ± 0.29	0.93 ± 0.08	0.94 ± 0.07	0.37 ± 0.05	0.00 ± 0.00	0.21 ± 0.06	0.42 ± 0.03	0.45 ± 0.03
ICGV 98324 x Tainan 9	0.91 ± 0.09	0.24 ± 0.36	0.50 ± 0.31	0.92 ± 0.08	0.96 ± 0.04	0.37 ± 0.05	0.20 ± 0.09	0.60 ± 0.10	0.36 ± 0.04	0.41 ± 0.04
Reduction¶										
ICGV 98308 x KK 60-3	0.79 ± 0.19	0.00 ± 0.00§	0.38 ± 0.33	0.85 ± 0.16	0.93 ± 0.11	0.30 ± 0.09	0.00 ± 0.08	0.11 ± 0.05	0.33 ± 0.08	0.37 ± 0.08
ICGV 98308 x Tainan 9	0.53 ± 0.27	0.00 ± 0.00	0.00 ± 0.00	0.72 ± 0.21	0.81 ± 0.16	0.21 ± 0.13	0.00 ± 0.00	0.00 ± 0.00	0.35 ± 0.16	0.37 ± 0.14
ICGV 98324 x KK 60-3	0.73 ± 0.20	0.21 ± 0.37	0.35 ± 0.33	0.83 ± 0.16	0.85 ± 0.15	0.37 ± 0.10	0.06 ± 0.07	0.11 ± 0.05	0.28 ± 0.09	0.31 ± 0.07
ICGV 98324 x Tainan 9	0.63 ± 0.24	0.00 ± 0.00	0.00 ± 0.00	0.80 ± 0.17	0.86 ± 0.14	0.40 ± 0.14	0.00 ± 0.00	0.00 ± 0.00	0.27 ± 0.12	0.31 ± 0.09
Correlation (r)†	0.77**	0.73**	0.81**	0.68**	0.67**	–	–	–	–	–

**Significant at 0.01 probability level.

†Correlations for traits related to NF between well-watered condition and drought conditions.

‡Standard error.

§Negative heritability estimates were assumed to be zero and expressed as zero.

¶Percentage of reduction (R) = [1− (trait under stress/trait under non stress)] x 100.

Table 3. Phenotypic (r_P) and genotypic (r_G) correlations between nitrogen fixation parameters [N_2 fixed (NF), nodule number (NN), nodule dry weight (NDW), shoot dry weight (SDW), total dry weight (TDW), and reduction (R) of these traits] and drought resistance traits [pod dry weight (PDW), reduction in pod dry weight (RPDW), harvest index (HI), specific leaf area (SLA), and SPAD chlorophyll meter reading (SCMR)] for 140 progeny lines of peanut under well-watered and drought stress conditions evaluated across two environments in dry season 2005/2006 and 2006/2007.

Traits		PDW		HI		SLA at 67 DAP¶		SCMR at 67 DAP		Traits		R§
		FC†	DSC‡	FC	DSC	FC	DSC	FC	DSC			PWD
NF	r_P	0.13	0.42**	−0.46**	−0.23**	0.00	0.13	0.22**	0.14	RNF	r_P	0.21*
	r_G	0.13	0.43**	−0.48**	−0.23**	0.02	0.14	0.23**	0.15		r_G	0.25**
NN	r_P	0.22**	0.32**	0.00	−0.08	0.30**	0.30**	0.08	0.04	RNN	r_P	−0.05
	r_G	0.27**	0.68**	−0.01	−0.20*	0.50**	0.70**	0.05	0.10		r_G	0.00
NDW	r_P	0.23**	0.39**	−0.10	−0.02	0.26**	0.22**	0.15	0.03	RNDW	r_P	−0.12
	r_G	0.26**	0.57**	−0.12	−0.01	0.38**	0.32**	0.14	0.04		r_G	−0.13
SDW	r_P	0.16	0.45**	−0.46**	−0.22**	0.04	0.18	0.21*	0.08	RSDW	r_P	0.22**
	r_G	0.15	0.47**	−0.46**	−0.20*	0.06	0.20*	0.20*	0.09		r_G	0.23**
TDW	r_P	0.60**	0.76**	0.01	0.16	0.00	0.22*	0.40**	0.14	RTDW	r_P	0.66**
	r_G	0.61**	0.78**	0.01	0.19	0.01	0.24**	0.41**	0.15		r_G	0.69**

*Significant at 0.05 probability level.

**Significant at 0.01 probability level.

†Field capacity (FC).

‡Drought stress conditions (DSC).

§Percentage of reduction (R) = [1− (trait under stress/trait under non stress)] x 100.

¶Days after planting (DAP).

and its related traits than under well-watered conditions. Because of their genetic correlation (r_G = 0.43, $P \leq 0.01$) selection for high NF in peanut could enhance pod yield under drought stress conditions. Pimratch et al. (2008) suggested that the ability to maintain high NF under drought stress could aid peanut genotypes in maintaining high yield under water-limited conditions. Similar results in soybean have been found by other researchers (Serraj and Sinclair, 1996; Patterson and Hudak, 1996). It is generally accepted that nitrogen is essential for growth and yield. Our results indicated that NF may be more important for yield under drought when N from soil is limited by water uptake.

The correlation coefficients between NF and its related traits and SCMR were not significant under water-stressed conditions and were lower than those under well-watered conditions (r_G = 0.04 to 0.15) (Table 3). This might be because higher chlorophyll concentration under drought conditions did not support photosynthesis due to other limitations. In relation to SLA, significant correlation coefficients were observed under drought conditions with NN, NDW, SDW, and TDW (r_G = 0.20, $P \leq 0.05$ to 0.70, $P \leq 0.01$) but not with NF (r_G = 0.14). This might suggest that leaf area is more important in maintaining photosynthesis under drought conditions and, thus, maintaining traits related to NF and biomass production. The lack of association between NF and SLA under drought might be due to low nitrogen use efficiency.

Under drought conditions, pod yield and NF are greatly reduced and the genotypes that can maintain reasonably high pod yield and NF are preferable. Selection for reduced rates of drought-induced reductions in these traits might be useful for improving drought resistance. In relation to reduction in pod yield, significant and positive correlation coefficients were found for reduction in NF (r_G = 0.25, $P \leq 0.01$), SDW (r_G = 0.23, $P \leq 0.01$), and TDW (r_G = 0.69, $P \leq 0.01$), whereas nonsignificant correlations were found for reduction in NN and NDW (Table 3). The results indicated that the reduction in pod yield was much closer to the reduction in TDW than NF and SDW. Selection for low reduction in TDW could be effective in producing genotypes with low reduction in pod yield.

CONCLUSION

Similar heritability estimates indicated that selection for NF and its related traits should be equally effective under well-watered and drought conditions. Selection for reduced rates of reduction in NF and related traits should be less effective because their heritability estimates were lower than heritabilities for the actual values. Nitrogen fixation was not correlated with PDW, but it was negatively correlated with HI, indicating that NF under well-watered conditions contributed to vegetative growth rather than to yield. However, selection for NF under drought might be more effective in improving yield under drought conditions because of a higher correlation than under well-watered conditions. The use of SCMR and SLA as surrogate traits for NF would be less effective than direct selection because of weak correlations. Selection for NF should have less significant effects on SLA and SCMR, but may significantly decrease HI. Selection for reduced rates of drought-induced reductions in NF, SDW, and TDW might be promising in regard to their high correlations with reduction of pod yield. However, these traits are not cost-effective, requiring evaluation under well-watered and drought conditions.

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Heritability of Drought Resistance Traits and Correlation of Drought Resistance and Agronomic Traits in Peanut

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ABSTRACT

Inheritance of traits is important for developing effective breeding schemes for improving desired traits. The aims of this study were to estimate the heritabilities (h^2) of drought resistance traits and the genotypic (r_G) and phenotypic (r_P) correlations between drought resistance traits and agronomic traits, and to examine the relationships between drought resistance traits under stressed and nonstressed conditions. The 140 lines in the $F_{4:7}$ and $F_{4:8}$ generations from four peanut (*Arachis hypogaea* L.) crosses were tested under field capacity (FC) and two-thirds available soil water (2/3 AW) in two field experiments. Data were recorded for specific leaf area (SLA), SPAD chlorophyll meter reading (SCMR), and biomass, pod yield, harvest index, number of mature pods per plant, seed per pod, and seed size. The h^2 for biomass, pod yield, DTI (drought tolerance index) (pod yield), DTI (biomass), HI, SLA, and SCMR were high for all tested crosses (0.54–0.98). The r_G (−0.61 and −0.66) and r_P (−0.61 and −0.66) between SLA and SCMR were strong and negative under 2/3 AW and FC. Under 2/3 AW conditions, SCMR was positively correlated with pod yield and seed size. Compared to SLA, SCMR had higher r_G and r_P with pod yield, biomass, and other agronomics traits. Significant correlations between FC and 2/3 AW conditions were found for pod yield, biomass, SCMR, and SLA, indicating that these traits could be selected under FC or 2/3 AW conditions. SPAD chlorophyll meter reading, which is easy to measure, is potentially useful as a selection trait for drought resistance because of high h^2 and positive correlation with pod yield and agronomic traits.

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Abbreviations: 2/3 AW, two-thirds available soil water; BIO, biomass; DAS, days after sowing; DTI, drought tolerance index; E, environment; FC, field capacity; G, genotype; HI, harvest index; PY, pod yield; SCMR, SPAD chlorophyll meter reading; SLA, specific leaf area; TE, transpiration efficiency; WUE, water use efficiency; Y, year.

DROUGHT IS THE major abiotic constraint affecting peanut (*Arachis hypogaea* L.) productivity and quality worldwide. Two-thirds of the global production occurs in rain-fed regions of the semi-arid tropics where rainfall is generally erratic and insufficient, causing unpredictable drought stress, the most important constraint for peanut production (Wright and Nageswara Rao, 1994; Reddy et al., 2003). Even peanut grown under irrigation may experience drought because of limited water supply or because irrigation water is applied in amounts at frequencies less than optimal for plant growth. Improving water access and management are practically difficult since water is a scarce resource. Therefore, breeding for drought resistance is an important strategy in alleviating the problem and offers the best long-term solution. Selection of segregating populations under stress conditions has been a standard approach for developing cultivars with improved stress tolerance. While direct selection for yield under stressed conditions can be effective, the limitations of this approach are high resource investment and poor repeatability of the results due to the large genotype ×

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environment (G×E) interaction that results in slow breeding progress (Wright et al., 1996).

More rapid progress may be achieved by using physiological traits (Nigam et al., 2005) such as harvest index (HI) or water use efficiency (WUE), specific leaf area (SLA), and SPAD chlorophyll meter reading (SCMR). Both SLA and SCMR have been used as surrogate traits for WUE (Wright et al., 1994; Nageswara Rao and Wright, 1994; Sheshshayee et al., 2006; Nigam et al., 2005). In a biological model, yield is explained to be a function of water transpired, WUE, and HI (ratio of economic yield to total biomass produced) (Passioura, 1986). Water use efficiency, defined as total biomass production per unit of water transpired, is not an easy trait to measure and, therefore, is not practical for use in large-scale breeding programs for improving drought tolerance.

Water use efficiency is negatively correlated with leaf carbon isotopic composition (Δ) in a range of crop species, including peanut (Farquhar et al., 1982; Hubick et al., 1986; Wright et al., 1988, 1994). While measurement of Δ is rapid, it is an expensive technique and may not be feasible in large segregating breeding populations, particularly in developing countries. Specific leaf area, the ratio of leaf area to leaf dry weight, is negatively related to leaf thickness and Δ and hence WUE, over a wide range of cultivars and environments in peanut (Wright et al., 1994; Nageswara Rao and Wright, 1994). Significant and high correlations between SLA and ribulose 1-5 bisphosphate carboxylase (Rubisco) (Nageswara Rao et al., 1995) suggested that photosynthetic capacity per unit leaf area is the major factor contributing to variation in WUE in peanut. There are a few published reports suggesting the predominant role of additive gene effects in SLA inheritance (Nigam et al., 2001; Surihan et al., 2005). Heritability estimation of water transpired, transpiration efficiency (TE), and HI has been reported that varied between crosses and traits (Cruickshank et al., 2004).

Nageswara Rao et al. (2001) reported significant correlations among SCMR, SLA, and specific leaf nitrogen. A strong and positive relationship between SCMR and WUE was found in peanut (Sheshshayee et al., 2006). Specific leaf area and SCMR are negatively correlated (Nageswara Rao et al., 2001; Upadhyaya, 2005). Upadhyaya (2005) also reported genetic variation for SCMR in peanut.

Information on the inheritance of HI, SLA, and SCMR and the genetic correlations among these traits will be useful for planning a suitable breeding strategy for improving drought tolerance. Drought can alter the heritability estimates of these traits; therefore, genetic gain through conventional selection may be different under drought and well-watered conditions. Genetic correlations between drought resistance traits and agronomic traits have to be studied in details under drought and well-watered conditions to evaluate correlated responses to selection of drought resistance traits on agronomic traits. Objectives of this study were to estimate (i) the heritabilities of drought resistance traits, (ii) the genotypic and phenotypic correlations between drought resistance traits and agronomic traits in peanut under different water levels, and (iii) the relationship between drought resistance traits under stressed and nonstressed condition.

MATERIALS AND METHODS

Genetics Materials

Four peanut F_1 hybrids (ICGV 98308 × 'KK60-3', ICGV 98324 × KK60-3, ICGV 98308 × 'Tainan 9', and ICGV 98324 × Tainan 9) were generated from the hybridization of two drought-resistant lines (ICGV 98308 and ICGV 98324; medium-maturing [110 d to maturity] and medium-seeded type), selected for low yield reduction, with two high-yielding cultivars, KK60-3 (late-maturing [120 d to maturity] and large-seeded type) and Tainan 9 (early-maturing [100 d to maturity] and medium-seeded type). ICGV 98324 and KK 60-3 are know to have high SCMR and low SLA, ICGV 98308 has moderate SLA and moderate SCMR, and Tainan 9 has high SLA and low SCMR under both stressed and nonstressed conditions. The F_1 seeds were planted and their seeds harvested in bulk for each cross. In the F_2 and F_3 generations, two pods were kept for each plant and bulked for each cross. Line separation was performed in the F_4 generation. A total of 140 lines (35 lines for each cross) were randomly selected and multiplied in the F_5 and F_6 generation.

The 140 families from four crosses were evaluated in the $F_{4:7}$ and $F_{4:8}$ generations (F_4–derived lines in the F_7 and F_8 generations, respectively) under two soil moisture levels, field capacity (FC) and two-thirds available soil water (2/3 AW), for 2 yr in dry season 2005–2006 and 2006–2007. 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 (16°28′ latitude, 102°48′ longitude, 200 m above sea level) during November 2005 to March 2006, and repeated during November 2006 to April 2007. Soil type is Yasothon series (loamy sand, Ocix Paleustults), with an FC soil moisture of 11.0% and permanent wilting point of 4.6%. Two soil moisture levels, FC (11.0%) and 2/3 AW (8.8%), in 0 to 60 cm depth were assigned as main plots, and peanut lines were laid out in subplots. Each entry was planted in five row plots 3.2 m long. Spacing was 50 cm between rows and 20 cm between hills within the row.

Crop Management

Land was prepared for planting by plowing three times. Lime (625 kg ha^{-1}), phosphorus fertilizer as triple superphosphate (24.7 kg P ha^{-1}), and potassium fertilizer as potassium chloride (31.1 kg K ha^{-1}) were applied before 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} seed before planting, and seeds of the large-seeded genotypes were also treated with ethrel (2-chloroethylphosphonic acid) 48% at the rate of 2 mL L^{-1} water to break dormancy. Three to four seeds were planted per hill, and the seedlings were thinned to two plants per hill at 14 d after sowing (DAS). Rhizobium was applied to the seed by applying a water-diluted commercial peat-based inoculum of *Bradyrhizobium* (mixture of strains THA 201 and THA 205; Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand) on the rows of peanut plants. Weeds were controlled by an application of alachlor [2-chloro-2',6'-diethyl-*N*-(methoxymethyl) acetanilide 48%, w v^{-1}, 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 45 DAS. 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^{-1}, water soluble concentrate] at 2.5 L ha^{-1}, methomyl [*S*-methyl-*N*-((methylcarbamoyl) oxy) thioacetimidate 40% soluble powder] at 1.0 kg ha^{-1} and carboxin [5,6-dihydro-2-methyl-1,4-oxathiine-3-carboxanilide 75% wettable powder] at 1.68 kg ha^{-1}.

A subsoil drip-irrigation system (Super Typhoon, Netafim Irrigation Equipment & Drip Systems, Israel), with a distance of 20 cm between emitters was installed with a spacing of 50 cm between drip lines at 10 cm below the soil surface midway between peanut rows and fitted with a pressure valve and water meter to ensure a uniform supply of measured amounts of water across each plot. Soil moisture was initially maintained at field capacity (102.63 mm in 60 cm depth) until 21 DAS in all treatments to support crop establishment. After 21 DAS, the 2/3 AW treatment was imposed by withholding irrigation until the soil moisture at 0 to 60 cm of soil depth was reduced to the predetermined levels of 82.57 mm at 60 cm depth. Afterward, soil moistures for the stress treatment was allowed to gradually decline until reaching the predetermined levels of 2/3 AW at 0 to 60 cm at 28 DAS, then held more or less constant until harvest. 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.

Total crop water use for each water treatment was calculated as the sum of transpiration and soil evaporation. Transpiration was calculated using the methods described by Doorenbos and Pruitt (1992):

$$ET_{crop} = ET_o K_c$$

where ET_{crop} is crop water requirement (mm d^{-1}), ET_o is evapotranspiration of a reference plant under specified conditions calculated by pan evaporation method, and K_c is the crop water requirement coefficient for peanut, which varies with genotype and growth stage (Doorenbos and Kassam, 1986). Surface evaporation (E_s) was calculated as (Singh and Russell, 1981)

$$E_s = \beta(E_o/t)$$

where E_s is soil evaporation (mm), β is light transmission coefficient measured depending on crop cover, E_o is evaporation from class A pan (mm d^{-1}), and *t* is days from the last irrigation or rain.

Data Collection

Weather Parameters

Weather data for both years were obtained from a meteorological station about 30 m away from the experimental site and are presented in Fig. 1.

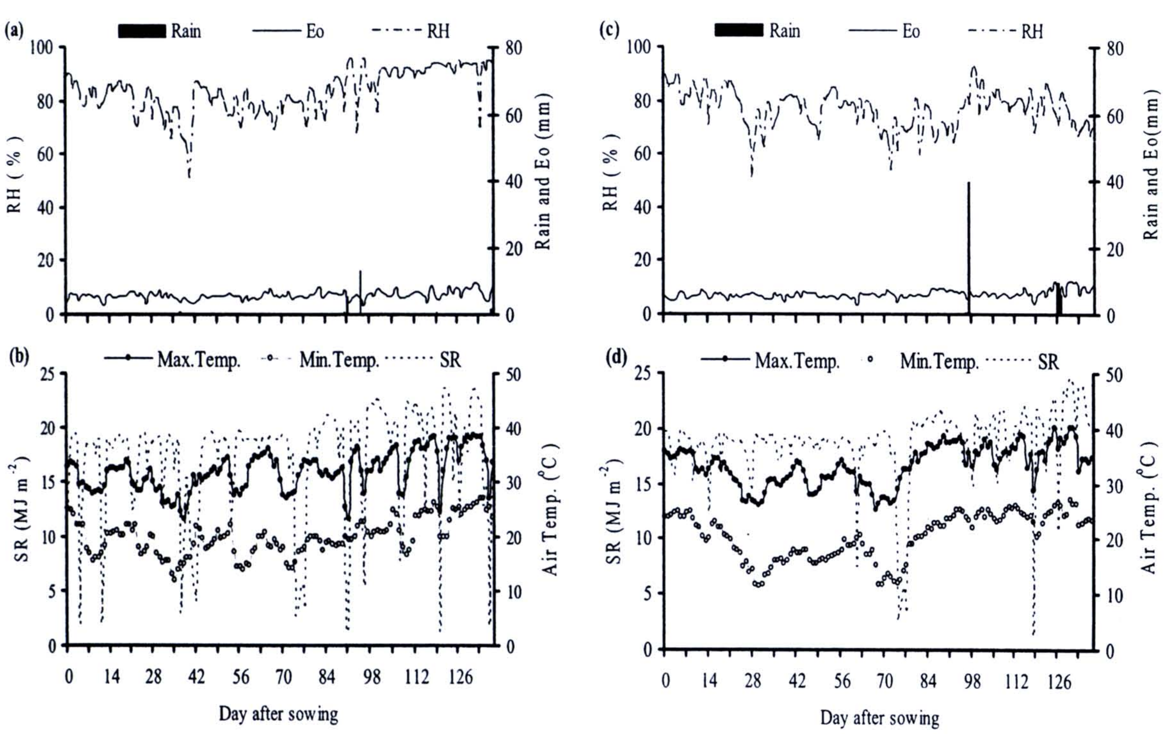

Figure 1. Rainfall, evaporation (E_O), relative humidity (RH), maximum and minimum air temperature, and solar radiation (SR) in (a, b) 2005–2006 and (c, d) 2006–2007 in Khon Kaen, Thailand.

The field trials were conducted during the dry seasons from November 2005 to March 2006 and November 2006 to April 2007. There was maximum rainfall (13.0 mm) at 95 DAS in the dry season 2005–2006, and (39 mm) at 97 DAS in the dry season 2006–2007 (Fig. 1). The seasonal mean maximum and minimum air temperature ranged between 32.0°C and 20.0°C in 2005–2006 and 33.0°C and 20.0°C in 2006–2007. Daily pan evaporation ranged from 2.8 to 9.6 mm in 2005–2006 and 2.9 to 9.8 mm in 2006–2007. Seasonal mean solar radiation was 16.7 MJ m^{-2} d^{-1} in 2005–2006 and, 18.8 MJ m^{-2} d^{-1} in 2006–2007.

Soil Moisture Status

Soil moistures were measured by the gravimetric method at planting and harvesting at the depths of 0 to 5, 25 to 30, and 55 to 60 cm. The measurement at planting was for calculating the correct amount of water to be applied to the crop, and the measurement at harvest was for calculating the water use of the crop. The soil water status was also monitored at 7-d intervals using a neutron moisture meter (Type I.H. II SER. No. N0152, Ambe Didcot Instruments Co., Abingdon, Oxon, UK). Sixteen-second neutron moisture meter readings were made at least weekly from a depth of 0.3 to 0.9 m at 0.3-m intervals.

SPAD Chlorophyll Meter Reading and Specific Leaf Area

In each plot, five plants were randomly selected to record SCMR and SLA at 52, 67, 82, and 97 DAS following the procedure described by Nageswara Rao et al. (2001). Briefly, the second fully expanded leaves were detached from the chosen plants between 8:30 and 9:30 a.m. and brought to the laboratory in zipped polythene bags for recording observations. The SPAD chlorophyll meter (Minolta SPAD-502 m, Tokyo, Japan) reading was recorded twice on each leaflet of the tetrafoliate leaf along the midrib. 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., Lincoln, NE) after which leaves were dried in an oven at 80°C for at least 48 h to determine the 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}). The SLA was calculated using the following formula: SLA = leaf area (cm^2)/leaf dry weight (g).

Agronomic Traits

For each plot, three rows with 2.8 m in length (4.2 m^2) were harvested at maturity (R8) (Boote, 1982), and their pods were removed before taking fresh shoot weight in the field. A 2-kg random sample of shoots was oven-dried at 80°C for 48 h and dry weight was measured. Shoot dry matter content was then calculated and used in determining shoot dry weight for a plot. Pod yields were weighed after air drying to approximately 8% moisture content.

The number of mature pods per plant (mature pods was separated from immature pods, which were identified by dark internal pericarp color), number of seed per pod and 100 seed weight were also recorded at final harvest.

Harvest index was computed by the following formula: HI = total pod weight at the final harvest/total biomass at the final harvest.

Drought tolerance index (DTI), as suggested by Nautiyal et al. (2002), was calculated for biomass—DTI (BIO)—and pod yield—DTI (PY)—as the ratio of each parameter under stressed treatments (2/3 AW) to that under well-watered (FC) condition.

Statistical Analysis

Individual analysis of variance was performed for each year followed a split-plot design (Gomez and Gomez, 1984). Homogeneity of variance was tested for all characters and combined analysis of variance of 2-yr data was performed. Calculation procedures were conducted using MSTAT-C package (Bricker, 1989). Because water regime × genotype interaction was significant, each water regime was analyzed separately according to a randomized complete block design (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 δ^2_G is genotypic variation, δ^2_P is phenotypic variation, r is number of replications, and e is number of environments. The standard error of heritability (Singh et al., 1993) for drought resistance traits was calculated to give a measure of the precision of the estimate.

Because the evaluation of heritability estimates was conducted in late generations (F_7 and F_8) 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 resistance traits and agronomic traits were calculated following the methods of Falconer and Mackay (1996) as follows (Table 1):

$$\text{Phenotypic correlation } (r_P) = (M^\star_3 M_3)/[(M^\star_3)(M_3)]^{1/2}$$

$$\text{Genotypic correlation } (r_G) = (M^\star_3 M_3 - M^\star_2 M_2)/[(M^\star_3 - M^\star_2)(M_3 - M_2)]^{1/2}$$

Table 1. Analysis of variance of cross and cross product.

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

†MCP, mean square of cross product; EMS, expected mean square; EMCP, expected mean square of cross product.

‡r, number of replications.

here M* is mean square of character X and M is mean square f character Y. Simple correlation was used to determine the elationship between biomass, pod yield, and drought resistance raits under well-watered and drought conditions to understand hether the performance of peanut genotypes was consistent cross environments.

ESULTS AND DISCUSSION

Monitoring of Soil Moisture

oil moisture was measured with a neutron moisture meter t 7-d intervals until harvest (Fig. 2). The results showed easonable management of soil moistures. A clear distinction among soil moisture levels was noted at 30 cm of soil depth. Soil moistures at 90 cm depth were similar among treatments because the amount of water applied in each treatment was calculated for 0 to 60 cm. Visual wilting was observed in the 2/3 AW treatment in the afternoon.

Combined Analysis of Variance

Combined analysis of variance showed significant differences among 140 progenies ($P \leq 0.01$) for biomass production, pod yield, and the drought surrogate traits HI, SCMR, and SLA (Table 2). This indicated that genetic variation exists for these characters and, thus, that heritability could

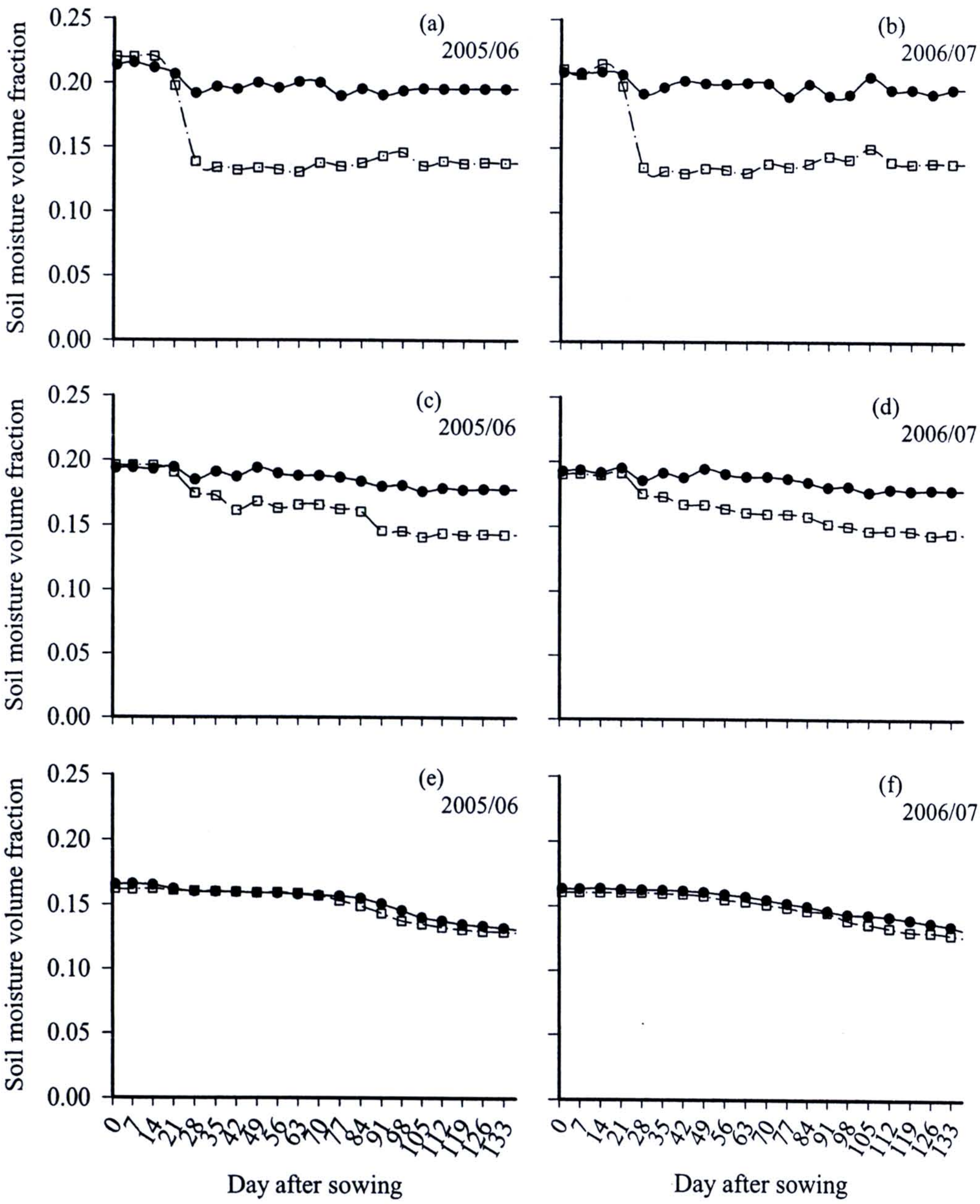

Figure 2. Soil moisture volume fraction in two available soil water regimes [field capacity (FC), ●; and 2/3 available water (AW), □] at (a, b) 30 cm, (c, d) 60 cm, and (e, f) 90 cm of the soil level during the 2005–2006 and 2006–2007 dry seasons in Khon Kaen, Thailand.

Table 2. Mean squares from the combined ANOVA for pod yield, biomass, and drought tolerance index for biomass, DTI (BIO),† and pod yield, DTI (PY), and harvest index (HI) at harvest, SPAD chlorophyll meter reading (SCMR), and specific leaf area (SLA) at 67 d after sowing under nonstressed (Non) and stressed (Stress) conditions of 140 peanut genotypes in the dry season of 2005–2006 and 2006–2007 in Khon Kaen, Thailand.

Source of variation	df	Pod yield			Biomass			HI		SCMR		SLA	
		Non	Stress	DTI (PY)	Non	Stress	DTI (BIO)	Non	Stress	Non	Stress	Non	Stress
Year (Y)	1	19.03	10.57**	0.17	209.41	39.11	0.49	0.00	0.04*	12.58	0.43	969.73	596.56
Rep. within Y	6	3.55	0.52	0.21	40.07	7.27	0.19	0.01	0.01	33.59	38.00	409.12	153.09
Genotypes (G)	139	2.95**	2.17**	0.39**	14.98**	14.60**	0.16**	0.03**	0.02**	57.48**	56.58**	124.53**	89.11**
Y × G	139	0.13**	0.10**	0.03	0.66	1.83	0.04	0.00	0.00*	2.79	2.48	11.68	5.17
Pooled error	834	0.09	0.07	0.03	0.78	1.73	0.04	0.00	0.00	4.08	3.58	14.04	7.77

*Significant at $P \leq 0.05$.

**Significant at $P \leq 0.01$.

†DTIs were calculated by the ratio of stressed (2/3 available water)/nonstressed (field capacity) conditions.

be estimated. The interaction effects of Y × G were significant ($P \leq 0.01$) for pod yield under well-watered and 2/3 AW conditions and significant ($P \leq 0.05$) for HI under 2/3 AW condition. In theory, pod yield is a complex trait in which multiple genes are involved, and high G × E interaction is expected (Wright et al., 1996). Based on low coefficient of variation and high *F*-ratio from analysis of variance, the best assessment times for SLA and SCMR was determined to be 67 DAS.

Heritability of Drought Resistance Traits

Heritability estimates within four peanut crosses were calculated for SCMR and SLA at 67 DAS, and for HI, DTI (BIO), and DTI (PY) at harvest (Table 3). Heritability estimates for HI, SLA, and SCMR were high for all four peanut crosses under both nonstressed and stressed conditions, ranging from 0.81 to 0.97. Drought tolerance indexes for pod yield and biomass showed lower heritability estimates than those for pod yield and biomass themselves under nonstressed and stressed conditions. Heritability estimates for BIO and PY varied from 0.73 to 0.98, and DTI (BIO) and DTI (PY) varied from 0.54 to 0.96.

Most characters had similar heritability estimates when compared between different water levels. This should make selection for drought tolerance easier. However, DTI is still useful in explaining how some genotypes had higher pod yield under drought. Previous reports on inheritance of drought resistance traits suggested a predominant role of additive gene effects in SLA and HI inheritance (Nigam et al., 2001; Surihan et al., 2005). In early generations (F_3 and F_4), Cruickshank et al. (2004) reported that broad-sense heritability of transpiration, TE, and HI were varied among peanut crosses and traits depending on levels of genetic variation in parents. Information on heritability of drought resistance traits [DTI (BIO), DTI (PY), HI, SCMR, and SLA)] under both stressed and nonstressed are needed for predicting progress from selection. Most of the drought resistance traits in our study had high heritability estimates, indicating that breeding progress could be achieved for these characters.

Table 3. Estimates of heritability with standard error for biomass (BIO), pod yield (PY), drought tolerance index for biomass, DTI† (BIO), and pod yield, DTI (PY), and harvest index (HI) at harvest and specific leaf area (SLA) and SPAD chlorophyll meter reading (SCMR) at 67 d after sowing of four crosses of peanut under stressed and nonstressed conditions in the dry seasons of 2005–2006 and 2006–2007 in Khon Kaen, Thailand.

Cross	Heritability						
	BIO	PY	DTI (BIO)	DTI (PY)	HI	SLA	SCMR
Stressed							
ICGV 98308 × 'KK60-3'	0.94 ± 0.06	0.93 ± 0.07	0.93 ± 0.07	0.86 ± 0.11	0.94 ± 0.05	0.93 ± 0.07	0.89 ± 0.10
ICGV 98308 × 'Tainan 9'	0.81 ± 0.16	0.95 ± 0.05	0.54 ± 0.25	0.92 ± 0.07	0.89 ± 0.08	0.81 ± 0.15	0.96 ± 0.03
ICGV 98324 × 'KK60-3'	0.73 ± 0.20	0.93 ± 0.07	0.67 ± 0.21	0.87 ± 0.11	0.95 ± 0.04	0.91 ± 0.08	0.92 ± 0.08
ICGV 98324 × 'Tainan 9'	0.96 ± 0.04	0.97 ± 0.03	0.86 ± 0.12	0.96 ± 0.03	0.89 ± 0.08	0.95 ± 0.05	0.96 ± 0.04
Nonstressed							
ICGV 98308 × 'KK60-3'	0.89 ± 0.12	0.91 ± 0.08	–	–	0.94 ± 0.04	0.83 ± 0.15	0.89 ± 0.11
ICGV 98308 × 'Tainan 9'	0.98 ± 0.02	0.98 ± 0.02	–	–	0.97 ± 0.02	0.91 ± 0.09	0.97 ± 0.02
ICGV 98324 × 'KK60-3'	0.93 ± 0.07	0.93 ± 0.06	–	–	0.92 ± 0.06	0.91 ± 0.09	0.90 ± 0.08
ICGV 98324 × 'Tainan 9'	0.98 ± 0.02	0.98 ± 0.01	–	–	0.96 ± 0.03	0.95 ± 0.05	0.96 ± 0.04

†DTIs were calculated by the ratio of stressed (2/3 available water)/nonstressed (field capacity) conditions.

Genotypic Correlation among Drought Resistance Traits

Phenotypic and genotypic correlations provided similar information in this study, and only genotypic correlations are reported. Strong and negative genotypic correlations were found between SLA and SCMR under both stressed and nonstressed conditions (−0.61, $P \leq 0.01$, and −0.66, $P \leq 0.01$, respectively) (Table 4). In previous studies, the simple correlation between SLA and SCMR was reported under nonstressed conditions (Wright et al., 1994; Nageswara Rao et al., 2001; Upadhyaya, 2005) and end-of-season drought conditions (Nigam and Aruna, 2008). In this study, we evaluated material in both stressed and nonstressed conditions in the same trials. Our finding show that genotypic and phenotypic correlations between SLA and SCMR were consistent under both FC and 2/3 AW conditions. The results show consistency of SLA and SCMR in a wide range of soil water levels and drought conditions. Drought tolerance index for pod yield had strong and positive genotypic correlation with DTI (BIO) (0.69, $P \leq 0.01$). Harvest index was quite low correlated with DTI (PY) under stressed condition (0.37, $P \leq 0.01$) and also was correlated with SCMR both under drought and well-watered conditions (0.13, $P \leq 0.01$, and 0.33, $P \leq 0.01$, respectively).

Genotypic Correlation between Drought Resistance Traits and Yield and Yield Components

Genetic correlations between drought resistance traits and yield and yield components provide information on expected responses in yield and yield components from selection for drought resistance traits. High genotypic correlations were found for HI and PY under drought (0.76, $P \leq 0.01$) and nonstressed (0.79, $P \leq 0.01$) conditions, and for HI with the number of mature pods per plant under both stressed and nonstressed treatments (0.62, $P \leq 0.01$, and 0.49, $P \leq 0.01$, respectively) (Table 5). The genotypic correlations between HI and seed size were also moderate and positive under both stressed and well-watered conditions (0.50, $P \leq 0.01$, and 0.47, $P \leq 0.01$, respectively). The surrogate traits for WUE (SLA and SCMR) (Wright et al., 1994; Nageswara Rao and Wright, 1994; Sheshshayee et al., 2006) had low correlation with pod yield. However, SCMR had higher genotypic correlations with PY, BIO, and other agronomic traits under both stressed and well-watered conditions than did SLA. SCMR showed quite low positive correlations with biomass (0.18; $P \leq 0.01$) and pod yield (0.21; $P \leq 0.01$) under stressed and moderate positive correlations with BIO (0.41; $P \leq 0.01$) and PY(0.51; $P \leq 0.01$) under well-watered conditions. SPAD chlorophyll meter reading was moderate positively correlated with seed size under stressed (0.43, $P \leq 0.01$) and well-watered (0.48; $P \leq 0.01$) conditions. DTI (BIO) and DTI (PY) had moderate positive correlations with biomass (0.47, $P \leq 0.01$, and 0.52, $P \leq 0.01$, respectively), with pod yield (0.34, $P \leq 0.01$, and 0.57, $P \leq 0.01$, respectively), and with number of mature pods per plant (0.34, $P \leq 0.01$, and 0.45, $P \leq 0.01$, respectively) under drought conditions. SPAD chlorophyll meter reading and SLA were strongly and negatively correlated at all evaluation dates (data not shown), and this association was relatively stable across environments (stressed and well-watered).

Among drought resistance traits [DTI (BIO), DTI (PY), HI, SCMR and SLA], HI had the highest correlation with PY, but the measurement of HI was more difficult, laborious, and costly than that of PY. Also, genetic correlations between SCMR and PY and HI were low. However, these traits have lower G × E interaction than do yield (Wright et al., 1996). It would be possible to

Table 4. Genotypic (r_G) correlation estimates among drought resistance traits for all four peanut crosses of 140 genotypes in the dry seasons of 2005–2006 and 2006–2007 in Khon Kaen, Thailand (df = 556).†

	Stressed				Nonstressed		
	DTI‡ (PY)	SCMR	SLA	HI	SCMR	SLA	HI
DTI (BIO)	0.69**	−0.34**	0.05	0.06	–	–	–
DTI (PY)		−0.28**	0.06	0.37**	–	–	–
SCMR			−0.61**	0.13**		−0.66**	0.33**
SLA				0.11*			−0.10*

*Significant at $P \leq 0.05$.

**Significant at $P \leq 0.01$.

†DTI, drought tolerance index; BIO, biomass, PY, pod yield, SCMR, SPAD chlorophyll meter reading; SLA, specific leaf area; HI, harvest index.

‡DTIS were calculated by the ratio of stressed (2/3 available water)/nonstressed (field capacity) conditions.

Table 5. Genotypic (r_G) correlation estimates between drought resistance traits and agronomic traits for all four peanut cross of 140 genotypes in the dry seasons of 2005–2006 and 2006–2007 in Khon Kaen, Thailand (df = 556).†

Drought resistance traits	Agronomic traits				
	BIO	PY	Seed size	No. mature pods/plant	Seed/pod
Stressed					
DTI‡ (BIO)	0.47**	0.34**	0.01	0.34**	0.29**
DTI (PY)	0.52**	0.57**	0.25**	0.45**	0.14**
SCMR	0.18**	0.21**	0.43**	−0.20**	−0.04
SLA	0.07	0.07	0.06	0.04	0.10*
HI	0.19**	0.76**	0.50**	0.62**	0.16**
Nonstressed					
SCMR	0.41**	0.51**	0.48**	0.02	0.24**
SLA	0.01	−0.09*	−0.12**	0.02	0.06
HI	0.01	0.79**	0.47**	0.49**	0.26**

*Significant at $P \leq 0.05$.

**Significant at $P \leq 0.01$.

†DTI, drought tolerance index; BIO, biomass, PY, pod yield, SCMR, SPAD chlorophyll meter reading; SLA, specific leaf area; HI, harvest index.

‡DTI were calculated by the ratio of stressed (2/3 available water)/nonstressed (field capacity) conditions.

improve yield by selecting for high HI and SCMR. The SCMR is an indicator of the photosynthetically active light-transmittance characteristics of the leaf and positive correlated with chlorophyll content (Akkasaeng et al., 2003) and chlorophyll density (Arunyanark et al., 2008) and WUE (Sheshshayee et al., 2006).

Nonetheless, the integration of physiological traits (or their surrogates) in the selection scheme would be advantageous in selecting genotypes that are more efficient water utilizers (SCMR [surrogates trait]) or partitioners of photosynthates into economic yield (HI) (Nigam et al., 2005). The SPAD chlorophyll meter provides an easy opportunity to integrate a surrogate measure of WUE with PY, in the selection scheme of a drought resistance breeding program in peanut.

Relationship of Drought Resistance Traits under Well-Watered versus Drought Conditions

A comparison of drought resistance traits under well-watered versus drought conditions should provided a better understanding of the most suitable conditions for selecting drought resistant genotypes. Significant correlations between traits under stressed and nonstressed conditions were found in all four peanut crosses for HI, SCMR, SLA, PY, and BIO (Table 6), indicating that theses traits could be selected either under well-watered or water-stressed conditions. As heritability estimates were high under both well-watered and stress conditions and the traits under different water regimes were correlated well, it is advisable to first select peanut genotypes under well-watered conditions in large early segregating populations because drought simulation is much more difficult; later, the selections can be refined under both drought and nonstressed conditions in advanced generations.

CONCLUSION

In summary, most traits measured in these four peanut crosses had high heritability, indicating that breeding progress should be possible. The results of the present study indicated that harvest index, SPAD chlorophyll meter reading, and specific leaf area observations can be recorded at both stressed and nonstressed conditions. This gives peanut breeders a large flexibility to record these observations in a large number of segregating populations and breeding lines in the field, thus making it easy to incorporate these physiological traits associated with drought tolerance in breeding and selection schemes in peanut. SPAD chlorophyll meter reading should be particularly useful as a selection criterion for drought tolerance in peanut because of high heritability and the simplicity in gathering.

Table 6. Correlation coefficients of biomass (BIO), pod yield (PY), harvest index (HI), specific leaf area (SLA), and SPAD chlorophyll meter reading (SCMR) of four peanut crosses under stressed (d) and well-watered (w) conditions during 2005–2006 and 2006–2007 in Khon Kaen, Thailand (df = 33).

Correlation	Peanut cross			
	ICGV 98308 × 'KK60-3'	ICGV 98308 × 'Tainan 9'	ICGV 98324 × 'KK60-3'	ICGV 98324 × 'Tainan 9'
BIO_w vs. BIO_d	0.48**	0.79**	0.62**	0.84**
PY_w vs. PY_d	0.35*	0.73**	0.61**	0.71**
HI_w vs. HI_d	0.75**	0.62**	0.58**	0.46**
$SCMR_w$ vs. $SCMR_d$	0.73**	0.84**	0.53**	0.86**
SLA_w vs. SLA_d	0.59**	0.35*	0.52**	0.86**

*Significant at $P \leq 0.05$.

**Significant at $P \leq 0.01$.

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Heritability of aflatoxin resistance traits and correlation with drought tolerance traits in peanut

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ABSTRACT

Drought tolerance can be an effective tool for improving aflatoxin resistance in peanut. We evaluated 140 peanut families from 4 crosses in the $F_{4:7}$ and $F_{4:8}$ generations in field trials under drought and non-drought conditions to investigate the heritability (h^2) of aflatoxin resistance traits, and phenotypic (r_P) and genotypic (r_G) correlations between aflatoxin resistance and drought tolerance traits. Data were collected for seed infection by *Aspergillus flavus*, aflatoxin contamination, biomass, pod yield, drought tolerance index (DTI) of biomass (BIO), DTI of pod yield (PY), harvest index (HI), SPAD chlorophyll meter reading (SCMR) and specific leaf area (SLA). High genotype x environment interactions and low to moderate h^2 for seed infection and aflatoxin contamination (0.30 to 0.65) were observed, indicating difficulty with directly improving these traits. However, seed infection and aflatoxin contamination had significant negative r_G with drought tolerance traits, especially with pod yield (−0.29** to−0.93**) under drought conditions, indicating that genotype selection for drought tolerance could improve aflatoxin resistance. Moreover, r_G were strong between seed infection and aflatoxin contamination with HI (−0.33** to−0.89**), SLA (0.50** to 0.92**) and SCMR (−0.60** to−0.83**). The results indicate that HI, SLA, or SCMR traits under long-term drought conditions could be used as indirect indicators of aflatoxin resistance. Because measurements for SLA and SCMR are simple, realizable and rapid, SLA and SCMR may be practical and cost effective for application in large scale breeding programs.

1. Introduction

Aflatoxin contamination is the most important quality problem for peanut (*Arachis hypogaea* L.) worldwide. While aflatoxin contamination affects peanut quality, the greater problem is that it affects the health of humans and livestock that consume contaminated peanut products. Avoidance of aflatoxin contamination through genetic manipulation has been a long-term goal of peanut breeders. However, resistant genotypes do not show consistency across different growing environments (Sanders et al., 1985; Kisyombe et al., 1985). A large genotype by environment (G x E) interaction for aflatoxin production hinders the progress of breeding programs. Moreover, the most resistant genotypes became susceptible to infection and subsequently contaminated by aflatoxin when they were grown under drought conditions (Blankenship et al., 1985; Anderson et al., 1995). In addition, aflatoxin resistance screening procedures have been dependent on expensive, laborious, and destructive techniques of directly measuring seed infection and aflatoxin content. Because of these constraints, there has been little improvement in the development of peanut genotypes with resistance to aflatoxin contamination.

Previous research suggested that tolerance to drought in peanut may be used as an indirect selection tool for resistance to pre-harvest aflatoxin contamination (Holbrook et al., 2000). Drought tolerant lines generally display lower levels of pre-harvest aflatoxin contamination, indicating that they may possess some degrees of resistance to aflatoxin contamination. Therefore, peanut breeding for drought tolerance might also be a promising strategy for improving resistance to aflatoxin contamination.

Breeding for resistance to drought based on yield is hindered by large G x E interactions (Jackson et al., 1996; Araus et al., 2002). Physiological and surrogate traits that are more stable than yield itself may be used as complementary tools for estimating yield under drought conditions. Such physiological characters as transpiration (T); transpiration efficiency (TE), defined as biomass produced per unit of water transpired; and harvest index (HI), the proportion of economic yield to total biomass, have been identified as traits associated with yield (Passioura, 1986). But these traits also have large G x E interactions, and are difficult to evaluate (Wright et al., 1994). More recent research in peanut has shown that TE

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is strongly correlated with easily measurable traits i.e., specific leaf area (SLA) and SPAD chlorophyll meter reading (SCMR) (Nageswara Rao and Wright, 1994; Arunyanark et al., 2008). These traits have been shown to be stable across environments and to have low G x E interactions (Nageswara Rao et al., 2001).

These surrogate traits for drought tolerance may also be applied for resistance to aflatoxin production. Holbrook et al. (2000) reported significant positive correlation between aflatoxin contamination and leaf temperature, and between aflatoxin contamination and visual stress ratings. However, these correlations were very small. More recently, combinations of drought tolerance traits SLA and root length density were strongly related with aflatoxin contamination ($r = 0.68^{**}$ to 0.75^{**}) under drought conditions (Arunyanark et al., 2009b). These traits could be good selection criteria for resistance to aflatoxin contamination. However, root length density is difficult to measure and as such is not practical in large-scale breeding programs. Conversely, SLA is simply method and it can be evaluated using SCMR (Nageswara Rao et al., 2001), therefore, SLA and SCMR may be used as a rapid, low-cost, and non-destructive technique to screen large breeding populations for resistance to aflatoxin contamination. However, although a correlation between SLA and SCMR has been established, a SCMR does not directly measure SLA. Moreover, the genetic relationships between SLA and SCMR with aflatoxin resistance traits were not investigated. SLA and SCMR will help to increase potential of breeding scheme if they are related with aflatoxin resistance.

Information on the inheritance of aflatoxin resistance traits and their correlations with other related traits will be useful for planning suitable breeding strategies for improving aflatoxin resistance. The heritability information under drought is very important because drought can increase the fungal infection and aflatoxin contamination that facilitates selection of low aflatoxin contamination. However, the inheritance of resistance to aflatoxin contamination is still lacking under drought conditions. Therefore, the objectives of this study were to evaluate (i) the heritability of aflatoxin resistance traits and (ii) phenotypic and genotypic correlations between aflatoxin resistance and drought tolerance traits under drought conditions.

2. Materials and Methods

2.1. Plant materials and population development

Four peanut F_1 hybrids (ICGV 98308 x KK 60-3, ICGV 98308 x Tainan 9, ICGV 98324 x KK 60-3, and ICGV 98324 x Tainan 9) were produced from the hybridization of two drought tolerant lines (ICGV 98308 and ICGV 98324) previously selected for low yield reduction under drought with two high yielding cultivars KK 60-3 (large seeded type) and Tainan 9 (medium seeded type) under non water stress conditions. ICGV 98324 is known to have low levels of seed infection by *A. flavus* and aflatoxin contamination, ICGV 98308 and KK 60-3 have moderate levels of seed infection and aflatoxin contamination, and Tainan 9 has high level of seed infection and aflatoxin contamination. The F_1 seeds were planted and harvested in bulk for each cross. In F_2 and F_3 generations, two pods from each plant were bulked for each cross. A total of 140 lines (35 lines for each cross) were randomly selected in the F_4 generation and the selections were advanced for two more generations (F_5 and F_6) for seed increase, so ample F_7 generation seeds were available for field trials.

2.2. Field trials

The 140 families from the 4 crosses in $F_{4:7}$ and $F_{4:8}$ generations (F_4-derived lines in the F_7 and F_8 generations, respectively) were evaluated in field tests under continuous long-term drought (2/3 available water) and non-drought (field capacity) conditions for two years in the dry seasons of 2005/06 and 2006/07. The four parents and six check varieties were included for comparison only. Field experiments were conducted at the Field Crop Research Station, Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen Thailand (16° 26″ N, 102° 50″ E) during November 2005 to March 2006, and November 2006 to April 2007. The soil type is Yasothon soil series (loamy-sand, Oxic Paleustults). The experiment was laid out in a split plot design with four replications with two water regimes (drought and control) as main plot treatments, and the genotypes as sub-plot treatments. Each entry was planted in a five-row sub-plot, with each sub-plot being 3.2 m long and having 50 cm between rows and 20 cm between hills within a row.

A subsoil-drip-irrigation system (Super Typhoon®, Netafim Irrigation equipment & Drip systems, Israel) was installed 10 cm below the soil surface mid-way between pairs of peanut rows and fitted with a pressure valve with a water meter to ensure a uniform supply of measured amounts of water to each main-plot, and the main-plots were separately controlled. To support crop establishment in all treatments, from 1 to 21 DAS, soil moisture was maintained at field capacity, which 103 mm to 60 cm depth, as determined using a pressure plate. After 21 DAS, the 2/3 available water treatment was imposed by withholding irrigation until the soil moisture at 0–60 cm soil depth was reduced to the predetermined levels of 83 mm in 60 cm depth. Soil moisture for the stress treatment was allowed to gradually decline until reaching the predetermined levels of 2/3 available water at 0–60 cm at 28 DAS, then were held more or less constant until harvest. The water amount was controlled only for main plot level. There were two main plots in a replication. To control water at genotype level, the experiment must be carried out to determine coefficient for each genotype, and this is not possible for a large population. Therefore, amount of water was uniform for all genotypes in each main plot. In order to maintain the specified soil moisture regimes, amounts of water were added to the respective main-plots based on crop water requirement and surface evaporation, which were calculated following the methods described by Doorenbos and Pruitt (1992) and Singh and Russel (1981), respectively.

Difference of 20 mm of soil moisture in FC (103 mm) and 2/3 AW (83 mm) can cause drought stress because root distribution is mainly on the top soil where soil moisture was very different between stressed and non-stressed treatments. Previous studies have found that differences in visual wilting observed between FC and 2/3 AW treatments. Moreover, relative water content (RWC) declined significantly from > 95% in FC to 85–90% in 2/3 AW treatments, leading to reductions in total dry matter and pod yield in the 2/3 AW treatments (Arunyanark et al., 2008). These were different between FC and 2/3 AW and can be used to evaluate the severity of drought stress. Moreover, genetic variation of peanut for aflatoxin contamination was high under 2/3 AW conditions (Arunyanark et al., 2009b). Therefore, 2/3 AW treatment could be used to evaluate aflatoxin resistance in peanut.

Plants could be less possibility to stress under soil moisture at FC if the climate was very high evaporation with high temperature and strong wind. These factors can cause stress because the difference between water loss and water uptake. However, this situation can occur at very short period of some day that it should not affects to the plants.

An aflatoxin-producing *A. flavus* strain isolated from peanut kindly provided by the laboratory of Suranaree University of Technology, Nakhonratchasima province, Thailand, was multiplied in a peanut-based culture medium to produce inoculum. Inoculum was incorporated in to the soil for all plots at 375 kg ha^{-1} at 10 cm depth shortly before planting to ensure the presence of sufficient aflatoxin producing fungi in the pod zone.

2.3. Meteorological data

Rainfall, relative humidity (RH), evaporation (E_0), maximum and minimum temperature, and solar radiation were recorded daily from sowing until harvest by a weather station located 30 m from the experimental field. The largest rainfalls were 13 mm at 95 DAS in 2005/06 and 39 mm at 97 DAS in 2006/07. The seasonal mean maximum and minimum air temperature ranged between 33 °C and 20 °C during the two seasons. Daily pan evaporation ranged from 2.8 to 9.6 mm in 2005/06 and from 2.9 to 9.8 mm in 2006/07. Seasonal mean solar radiation was 16.7 MJ m^{-2} d^{-1} in 2005/06 and 18.8 MJ m^{-2} d^{-1} in 2006/07.

2.4. Soil moisture

Soil moisture was measured gravimetrically at planting and at harvest for three soil layers: 0 to 5 cm, 25 to 30 cm, and 55 to 60 cm. Ten points randomly distributed in each main-plots were sampled. Measured soil moisture content was used to calculate the correct amount of water to be applied to the crop and to calculate crop water used. Weekly measurements of soil moisture status were also done with a neutron moisture meter (Type I.H. II SER. N° N0152, Ambe Didcot Instruments Co. Ltd., Abingdon, Oxon, UK) at the depths of 30, 60 and 90 cm. Ten tubes were installed permanently between peanut rows in each main-plots.

2.5. SPAD chlorophyll meter reading (SCMR) and specific leaf area (SLA)

Data were recorded for SCMR and SLA in both well-watered and drought treatments at 52, 67, 82 and 97 days after sowing (DAS) following the method described by Nageswara Rao et al. (2001). Five plants of each plot were randomly sampled and the penultimate fully-expanded leaf of each main stem was used for SPAD readings (Minolta SPAD-502 meter, Tokyo, Japan), assessing relative chlorophyll density, between 9.00–10.00 a.m. on the four leaflets of each tetra-foliate leaf of each of the 5 sampled plants. Data reported for each plot are averages of five plants.

After measuring SCMR, all the same leaves were detached to measure leaf area with an automatic leaf area meter (LI 3100C Area Meter, LI-COR Inc., USA). Leaves were then oven dried until constant weight was reached (at 80 °C for 48 hours) and weighed. SLA was calculated as the ratio of leaf area to leaf dry weight (cm^2 g^{-1}).

2.6. Biomass and pod yield

At maturity, above-ground biomass and pods were harvested from 2.8 m of the three center rows of each plot for a sample harvest area of 4.2 m^2. Fresh weights excluding roots were recorded in the field, and 2 kg sub-samples of total fresh weight were taken from the plots, oven-dried, and weighed. Biomass per plot was computed using fresh and dry weight ratio of the sub-sample and the total fresh weights. Pod yield was based on weight of the pods dried to approximately 8% moisture content.

Harvest index (HI) was computed as:

$$HI = \frac{\text{Total pod weight at harvest}}{\text{Total biomass at the final harvest}}.$$

Drought tolerance index (DTI), as suggested by Nautiyal et al. (2002), was calculated for biomass (BIO), and pod yield (PY) as the ratio of each parameter in the stress treatment (2/3 available water) to well-watered (field capacity) treatment.

2.7. Seed infection by A. flavus and aflatoxin contamination

Data on seed infection and aflatoxin contamination were collected only for the drought treatment. At harvest, 100 mature pods were selected randomly and shelled. From these samples, *A.flavus* infection was measured for 100 seeds. Seeds were surface sterilized for 3 min in 10% aqueous solution of Clorox (0.525% NaOCl) and rinsed three times with autoclaved water and placed on a moistened pre-sterilized paper towel in sterile plastic box. After incubation for 7 days at 25 °C, seeds infected by *A. flavus* (having a greenish mold on the seed coat) were counted.

Aflatoxin contamination was measured for a 100 g seed sample of seeds from mature pods after grinding in electric mill. For each assay, a sub-sample of 20 g meal was used for aflatoxin extraction in 100 ml of methanol–dimethyl formamide – water solution (70:1:29% v/v), homogenized at a high speed for 2 minutes and allowed to settle for 10 minutes. The supernatant was used for aflatoxin B_1 analysis using a modified, direct competitive ELISA (Enzyme Linked Immunosorbent Assay) (Chu et al., 1987; Chu, 1989). In each test, samples were assayed in duplicate wells. Concentration of aflatoxin B_1 in the sample was determined by comparing its absorbance with those of the standard samples. Standard sample dilutions of 500, 250, 125, 62.5, 31.2, 15.6, 7.8, and 0 ppb were used to construct a calibration curve.

2.8. Statistical analysis

Individual analysis of variance for each year was performed using a randomized complete block design (RCBD), because seed infection and aflatoxin contamination were recorded only for the drought treatments. The homogeneity of error variances was tested for seed infection and aflatoxin contamination and then combined analysis of variance of two-year data was performed where error variances for the two years were homogeneous.

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):

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

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

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 (years). The standard error (SE) of heritability (Singh et al., 1993) for aflatoxin resistance traits was calculated to measure the precision of the heritability estimate.

As the evaluation of heritability estimates was conducted in late generations (F_7 and F_8) 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 aflatoxin resistance and drought tolerance traits were calculated based on family means (35 families of each cross) by the method described by Pimratch et al. (2009) and Songsri et al. (2008) as follows (Table 1).

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

where M_2 = mean square of families x years of character *X*, M_3 = mean square of families of character *X*, M_2^* = mean square of families x years of character *Y* and M_3^* = mean square of families of character *Y*.

Table 1
Outline of the combined analysis of variance and covariance over Y years, based on randomized complete block design (RCBD) with F families and r replications.

Sours of variance	df	Mean square of Character		MCP[†]	EMS[†]	EMCP[†]
		X	Y			
Years (Y)	Y−1					
Rep.within Y	Y(r−1)[‡]					
Families (F)	F−1	M_3^*	M_3	$M^*_3\ M_3$	$\sigma^2_E + r\sigma^2_{FE} + re\sigma^2_F$	$\sigma_{E^*E} + r\sigma_{FE^*FE} + re\sigma_{F^*F}$
F x Y	(F−1)(Y−1)	M_2^*	M_2	$M^*_2\ M_2$	$\sigma^2_E + r\sigma^2_{FE}$	$\sigma_{E^*E} + r\sigma_{FE^*FE}$
Pooled error	Y(r−1)(F−1)	M_1^*	M_1	$M^*_1\ M_1$	σ^2_E	σ_{E^*E}

[†] MCP, mean square of cross product; EMS, expected mean square; EMCP, expected mean square of cross product.
[‡] r, number of replication.

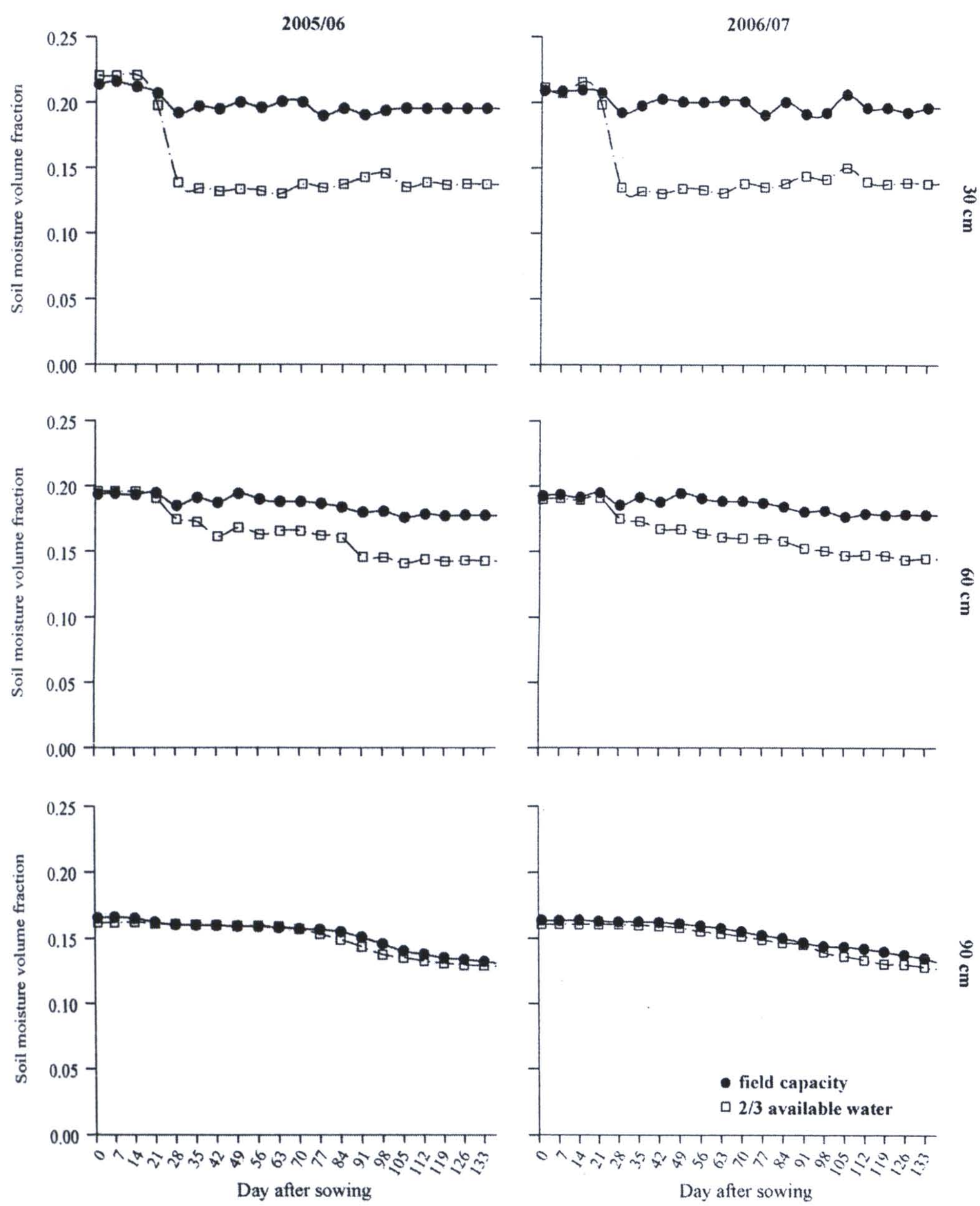

Fig. 1. Soil moisture volume fraction in two soil water regimes (field capacity and 2/3 available water) at 30, 60 and 90 cm of the soil level during the 2005/06 and 2006/07 dry seasons.

Table 2
Pooled analysis of variance over two years of seed infection and aflatoxin contamination under drought stressed conditions of 140 genotypes in dry seasons, 2005/06 and 2006/07.

Source of variance	df	Mean squares	
		Seed infection	Aflatoxin contamination
Year (Y)	1	249257**	5869
Rep. within Y	6	2665	1594
Genotypes (G)	139	519**	893**
G x Y	139	308**	471**
Pooled error	834	225	292

**Significant at $P \leq 0.01$.

3. Results

3.1. Soil moisture status

Soil moisture content clearly differed between water regimes, soil moistures in the drought treatment were lower than those of the irrigated treatment (Fig. 1). A clear distinction for both soil moisture levels was noted at 30 and 60 cm soil depths. Soil moisture at 90 cm depth was similar among treatments because the amount of water applied in each treatment was calculated for 0–60 cm layer. Wilting in the drought treatments was visually observed in the afternoons. In generally, wilting can be observed in 2/3 available water plots at early afternoon on the other day after irrigation but the plants can recover in the evening. The wilting can be severe with days after watering until the next watering. The severity can be indicated by wilting appearance and duration. However, wilting differences between genotypes were not determined.

3.2. G x E interaction for aflatoxin resistance traits

Combined analysis of variance across two years showed significant year effect for seed infection (Table 2). Peanut progenies (genotypes) were also significantly different ($P \leq 0.01$) for seed infection and aflatoxin contamination, indicating genetic variations for these characters. However, G x Y interactions were also significant ($P \leq 0.01$) for seed infection and aflatoxin contamination indicating higher environmental (year) effect.

3.3. Heritability of aflatoxin resistance traits

Heritability estimates for seed infection by *A. flavus* and aflatoxin contamination were low to moderate for all crosses (Table 3).

Table 3
Estimates of heritability with standard error for seed infection and aflatoxin contamination under drought conditions of 4 crosses in dry seasons, 2005/06 and 2006/07.

Cross	Heritability	
	Seed infection	Aflatoxin contamination
ICGV 98308 x KK 60-3	0.30 ± 0.50	0.30 ± 0.49
ICGV 98308 x Tainan 9	0.51 ± 0.34	0.61 ± 0.25
ICGV 98324 x KK 60-3	0.41 ± 0.40	0.56 ± 0.33
ICGV 98324 x Tainan 9	0.51 ± 0.33	0.65 ± 0.22

3.4. Phenotypic and genotypic correlation between drought tolerance and aflatoxin resistance

Correlations between drought tolerance traits and traits related to aflatoxin contamination are presented in Table 4. Genotypic correlations in general were slightly higher than phenotypic correlations in both negative and positive directions. Most correlations were negative and significant. However, most correlations were low and not consistent in different crosses except for the genotypic correlations between pod yield with seed infection (r_G = −0.49** to −0.93**) and aflatoxin contamination (r_G = −0.31** to −0.56**) which were significant for all crosses. The consistency of the correlations indicates the possibility to selecting of high-yielding genotypes with low seed infection and aflatoxin contamination.

3.5. Phenotypic and genotypic correlation between drought surrogate traits (HI, SLA and SCMR) with aflatoxin resistance

Again, genotypic correlations in general were higher than phenotypic correlations for all combinations of characters and for both negative and positive directions (Table 5). The correlations between HI, SLA, and SCMR with seed infection were similar to the correlations between these characters with aflatoxin contamination. These correlations were significant for all crosses. HI and SCMR were significantly correlated with seed infection and aflatoxin contamination, but the direction of the correlations was negative. The results suggest that selection for high HI and SCMR could lower seed infection and aflatoxin contamination. In contrast to HI and SCMR, the correlations between SLA with seed infection and aflatoxin contamination were all positive and significant. This result suggests that peanut genotypes with small SLA, that is, thick leaves, should be selected in breeding programs that aim to reduce seed infection and aflatoxin contamination. It is important to note here that the correlations of SCMR and SLA were much higher than those of HI. The similar results of phenotypic and genotypic correlations between SLA and SCMR with seed infection and aflatoxin contamination were also found at 52, 82 and 97 DAS (data not shown).

Table 4
Genotypic (r_G) and phenotypic (r_P) correlation between seed infection and aflatoxin contamination with biomass, pod yield, and drought tolerance index for biomass (DTI† (BIO)) and pod yield (DTI (PY)) of 4 crosses under drought conditions, 2005/06 and 2006/07.

	Biomass		Pod yield		DTI (BIO)		DTI (PY)	
	r_P	r_G	r_P	r_G	r_P	r_G	r_P	r_G
Seed infection								
ICGV 98308 x KK 60-3	−0.14	−0.15	−0.31**	−0.49**	−0.26**	−0.42**	−0.30**	−0.51**
ICGV 98308 x Tainan 9	−0.53**	−0.86**	−0.61**	−0.93**	0.10	0.28**	−0.54**	−0.76**
ICGV 98324 x KK 60-3	−0.08	−0.22**	−0.41**	−0.63**	0.18*	0.16	−0.22**	−0.27**
ICGV 98324 x Tainan 9	−0.42**	−0.62**	−0.59**	−0.86**	−0.04	−0.09	−0.38**	−0.58**
Aflatoxin contamination								
ICGV 98308 x KK 60-3	0.11	0.30**	−0.18*	−0.31**	−0.12	−0.19*	−0.28**	−0.55**
ICGV 98308 x Tainan 9	−0.35**	−0.54**	−0.37**	−0.46**	0.01	−0.05	−0.12	−0.17*
ICGV 98324 x KK 60-3	−0.06	0.11	−0.21*	−0.29**	−0.20*	−0.16	−0.38**	−0.55**
ICGV 98324 x Tainan 9	−0.34**	−0.45**	−0.44**	−0.56**	−0.20*	−0.32**	−0.34**	−0.43**

*,**Significant at the 0.05 and 0.01 probability levels, respectively. †DTI were calculated by the ratio of stressed (2/3 available water)/non-stressed (field capacity) conditions.

Table 5
Genotypic (r_G) and phenotypic (r_P) correlation between seed infection and aflatoxin contamination with harvest index (HI) at final harvest, specific leaf area (SLA) and SPAD chlorophyll meter readings (SCMR) at 67 DAS of 4 crosses under drought conditions, 2005/06 and 2006/07.

	HI		SLA		SCMR	
	r_P	r_G	r_P	r_G	r_P	r_G
Seed infection						
ICGV 98308 x KK 60-3	-0.19*	-0.34**	0.45**	0.75**	-0.35**	-0.60**
ICGV 98308 x Tainan 9	-0.42**	-0.60**	0.39**	0.68**	-0.43**	-0.62**
ICGV 98324 x KK 60-3	-0.46**	-0.71**	0.36**	0.55**	-0.45**	-0.67**
ICGV 98324 x Tainan 9	-0.56**	-0.89**	0.39**	0.50**	-0.54**	-0.77**
Aflatoxin contamination						
ICGV 98308 x KK 60-3	-0.20*	-0.41**	0.44**	0.73**	-0.48**	-0.83**
ICGV 98308 x Tainan 9	-0.27**	-0.33**	0.55**	0.68**	-0.50**	-0.63**
ICGV 98324 x KK 60-3	-0.23**	-0.37**	0.66**	0.92**	-0.59**	-0.83**
ICGV 98324 x Tainan 9	-0.45**	-0.53**	0.58**	0.70**	-0.63**	-0.79**

*,** Significant at the 0.05 and 0.01 probability levels, respectively.

4. Discussion

Drought conditions favor the productions of aflatoxin, drought levels should be adequately high to assess peanut genotypes resistant to aflatoxin contamination (Anderson et al., 1996; Holbrook et al., 1994). Adequately severe stress level is drought levels which it can increase the fungal infection and aflatoxin contamination. And, the stress should promote maximum variation of genotypes for aflatoxin contamination that facilitates selection of low aflatoxin contamination. Previous studies have found that drought stress at 2/3 AW was high seed infection and aflatoxin contamination, and high genotypes variation for aflatoxin contamination (Arunyanark et al., 2009b). Therefore, in this study, data of seed infection and aflatoxin contamination were recorded only under the 2/3 AW treatment.

Measurement of aflatoxin contamination is far more expensive and laborious that observing seed infection. Therefore, seed infection could be a good clue of aflatoxin contamination but it is not sufficient to assess aflatoxin resistance because of inconsistent relationship between these characters depending on environments and mould strains.

The high G x E interactions for seed infection by *A. flavus* and aflatoxin contamination in this study were not unexpected because G x E interaction has long been recognized as the main reason for the lack of consistency of the performance of peanut genotypes for aflatoxin contamination. Moreover, our results indicated that the heritability estimates for seed infection and aflatoxin contamination were rather low. These results support the fact that aflatoxin contamination and seed infection are complex traits and labile to environmental variations (Sanders et al., 1993; Anderson et al., 1995), and, thus, breeding for aflatoxin resistance is difficult to achieve without using more effective tools.

Drought tolerance traits might be useful as indirect selection tools for pre-harvest aflatoxin contamination resistance. Our results were in good agreement with the previous studies, which suggested that drought tolerant genotypes had greatly reduced aflatoxin contamination when grown under drought conditions (Holbrook et al., 2000).

Breeding of peanut for resistance to drought has been based on the ability of genotypes to maintain yield productivity under drought conditions. In this study, we found evidence that high pod yield under drought conditions is related to low seed infection and low aflatoxin contamination, indicating the possibility to select peanut lines with high pod yield and low aflatoxin contamination under drought conditions. We also found a close association between biomass production and drought tolerance indices of biomass and pod yield with seed infection and aflatoxin contamination. These observations provide evidence that breeding for drought tolerance could also improve aflatoxin resistance.

Moreover, in a parallel study in the same materials, heritability estimates were reported for biomass ($h^2 = 0.73$ to 0.96), pod yield ($h^2 = 0.93$ to 0.97), and DTI of biomass ($h^2 = 0.54$ to 0.93) and pod yield ($h^2 = 0.86$ to 0.96) (Songsri et al., 2008), and these were relatively higher than those for seed infection ($h^2 = 0.30$ to 0.51) and aflatoxin contamination ($h^2 = 0.30$ to 0.65) (Table 3). Because of higher heritabilities, selection for higher yield or higher biomass production should be easier than selection for aflatoxin resistance. However, high G x E interactions for pod yield were also reported (Songsri et al., 2008). High G x E interaction causes difficulty in identifying superior genotypes for yield and aflatoxin resistance and also leads to slow progress of breeding programs.

More rapid breeding progress may be achieved by using physiological or surrogate traits, such as HI, SLA, and SCMR, which may be used to select for drought tolerance (Wright et al., 1994; Sheshshayee et al., 2006; Arunyanark et al., 2009a). Furthermore, it might be possible to use these traits as indirect selection tools for resistance to aflatoxin contamination. Arunyanark et al. (2009b) observed that the combination of SLA and root length density was strongly related with aflatoxin contamination. Surrogate traits of drought tolerance might be used for selection of aflatoxin resistance. While the formation of indices with several traits is not suitable for breeding, simple indices are more advantageous. To the best of our knowledge, this is the first report showing evidence of the genetic relationship between the simple traits of HI, SLA, and SCMR with seed infection and aflatoxin contamination. These relationships were also consistent in different populations in this study. The results indicated that a breeding program to develop high HI, low SLA or high SCMR genotypes can also improve resistance to seed infection and aflatoxin contamination.

SLA and SCMR have been shown to be stable across environments due to low G x E interactions (Arunyanark et al., 2008; Nageswara Rao et al., 2001). In a parallel study with the same materials, HI had lower G x E interactions than did pod yield, and SLA and SCMR exhibited no G x E, showing stability of these traits across environments. They also had high heritability estimates for both non-drought and drought conditions, ranging from 0.89 to 0.97 for HI, ranging from 0.81 to 0.95 for SLA, ranging from 0.89 to 0.97 for SCMR (Songsri et al., 2008). On the basis of higher heritability and lower G x E interaction, more breeding progress for aflatoxin resistance may be achieved using the traits, SLA, SCMR and HI than using yield.

The experiment was conducted under stressed and non-stressed conditions. DTI was calculated for yield and biomass only. SCMR and SLA were also evaluated under both conditions. However, SCMR and SLA under well-watered conditions were reported in a separate paper (Songsri et al., 2008). SCMR and SLA are not necessary to evaluate under two conditions and only drought conditions is sufficient because G x E interactions were not significant, but evaluation at two conditions is necessary for yield and biomass.

Due to the stronger relationships of SLA and SCMR to aflatoxin resistance than other traits, these traits had high potential in breeding programs for aflatoxin resistance. Moreover, SLA and SCMR are very simple methods. Thus, either SLA or SCMR may be practical and cost effective for application in large scale breeding programs. SCMR is highly recommended to use because it is simpler method than SLA if a tool is available.

Our study was conducted under a long-term drought. However, the most critical period to aflatoxin contamination is late season drought (Sanders et al., 1985). Further investigations on the inheritance of aflatoxin resistance and phenotypic and genetic correlations between drought tolerance and aflatoxin contamination under late season drought are still required.

In summary, high G x E interaction and low heritability of aflatoxin resistance traits confound the selection of superior genotypes, and there is a need to develop alternative selection strategies. Genetic relationships between aflatoxin resistance traits and drought tolerance traits, especially pod yield under drought, have been established in this study. There is a high possibility to simultaneously improve pod yield and aflatoxin resistance under drought conditions in peanut populations evaluated in this study. Surrogate traits for drought tolerance, namely HI, SLA, and SCMR were closely related with low seed infection and low aflatoxin contamination. Therefore, they could be used as indirect selection tools for resistance to aflatoxin contamination.

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Heritability of, and genotypic correlations between, aflatoxin traits and physiological traits for drought tolerance under end of season drought in peanut (*Arachis hypogaea* L.)

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ABSTRACT

More rapid progress in breeding peanut for reduced aflatoxin contamination should be achievable with a better understanding of the inheritance of, aflatoxin trait and physiological traits that are associated with reduced contamination. The objectives of this study were to estimate the heritability of aflatoxin traits and genotypic (r_G) and phenotypic (r_P) correlations between drought resistance traits and aflatoxin traits in peanut. One hundred-forty peanut lines in the $F_{4:6}$ and $F_{4:7}$ generations were generated from four crosses, and tested under well-watered and terminal drought conditions. Field experiments were conducted under the dry seasons 2006/2007 and 2007/2008. Data were recorded for biomass (BIO), pod yield (PY), drought tolerance traits [harvest index (HI), drought tolerance index (DTI) of BIO and PY, specific leaf area (SLA), and SPAD chlorophyll meter reading (SCMR)], and aflatoxin traits [seed infection and aflatoxin contamination]. Heritabilities of *A. flavus* infection and aflatoxin contamination in this study were low to moderate. The heritabilities for seed infection and aflatoxin contamination ranged from 0.48 to 0.58 and 0.24 to 0.68, respectively. Significant correlations between aflatoxin traits and DTI (PY), DTI (BIO), HI, biomass and pod yield under terminal drought conditions were found (r_P = −0.25** to 0.32**, r_G = −0.57** to 0.53**). Strong correlations between SLA and SCMR with *A. flavus* infection and aflatoxin contamination were also found. Positive correlations between SLA at 80, 90, and 100 DAP and aflatoxin traits were significant (r_P = 0.13** to 0.46**, r_G = 0.26** to 0.81**). SCMR was negatively correlated with aflatoxin traits (r_P = −0.10** to −0.40**, r_G = −0.11** to −0.66**). These results indicated that physiological-based selection approaches using SLA and SCMR might be effective for improving aflatoxin resistance in peanut.

1. 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 × environment (G × 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, 1996; Holbrook et al., 1994). 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.

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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., 2009; Girdthai et al., 2010; Holbrook et al., 2000). However, improvement of drought resistance based on yield is also hindered by high G × E interactions (Jackson et al., 1996; Araus et al., 2002). Drought resistance traits with lower G × 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. (2009) 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. (2010) 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 under drought conditions. Arunyanark et al. (2010) 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.

2. Materials and methods

2.1. 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 and SLA with high pod yield and SCMR under drought stress (Girdthai et al., 2010). KK 60-3 [late maturing (120 days to maturity) and large seeded type] selected for high PAC, SCMR and biomass with low SLA, and Tainan 9 [early maturing (100 days to maturity) and medium seeded type] selected for high PAC and SCMR with low SCMR and biomass (Girdthai et al., 2010; Puangbut et al., 2009; Songsri et al., 2009) are released cultivars and widely grown in Thailand. Four F_1 hybrids (ICGV 98348 × KK 60-3, ICGV 98348 × Tainan 9, ICGV 98353 × KK 60-3, and ICGV 98353 × 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.

Four parental lines and the 140 progenies 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 2006/2007 and repeated in the dry season 2007/2008. 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.

2.2. 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}.

2.3. 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.

2.4. *A. flavus inoculation*

Inoculum of *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 transfer to peanut-based medium (ground peanut seeds and pods) and incubated at 25–30 °C for 14 days before cultivation. Ground peanut seeds and pods grown with *A. flavus* at rate of 375 kg ha^{-1} were broadcasted to peanut plots at 30 DAP.

2.5. *Data collection*

2.5.1. *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. 40 mm of the total amount of rainfall was recorded during 80–100 DAP in 2006/2007, and 22.7 mm was recorded during the same period in 2007/2008 (Fig. 1). Air temperature, relative humidity and evaporation in 2006/2007 were higher than in the 2007/2008, especially during the water stress period. During stress period (80 DAP to final harvest), mean evaporation was 6.0 and 5.0 mm in 2006/2007 and 2007/2008, respectively. The maximum and minimum air temperature ranged from 11.8 to 38.5 °C in 2006/2007 and 14.5 to 35.2 °C in 2007/2008, being lower during 80–110 DAP in 2007/2008. Relative humidity ranged from 54% to 93% in 2006/2007 and from 57% to 92% in 2007/2008. The seasonal mean solar radiation was 0.13 and 0.11 Cal cm^{-2} in 2006/2007 and 2007/2008, respectively.

2.5.2. *SPAD chlorophyll meter reading and specific leaf area*

Data were recorded for SCMR and SLA at 80, 90, and 100 DAP when crop exposed to terminal drought stress following by Girdthai et al. (2010). 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 a.m. 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 × 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 h 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 ($cm^2\ g^{-1}$).

2.5.3. *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 h 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:

$$HI = \frac{\text{pod weight}}{\text{total biomass}} \tag{1}$$

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).

2.5.4. *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 ELISA (Enzyme Linked Immunosorbent Assay) method slightly 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 min. 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 min 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.

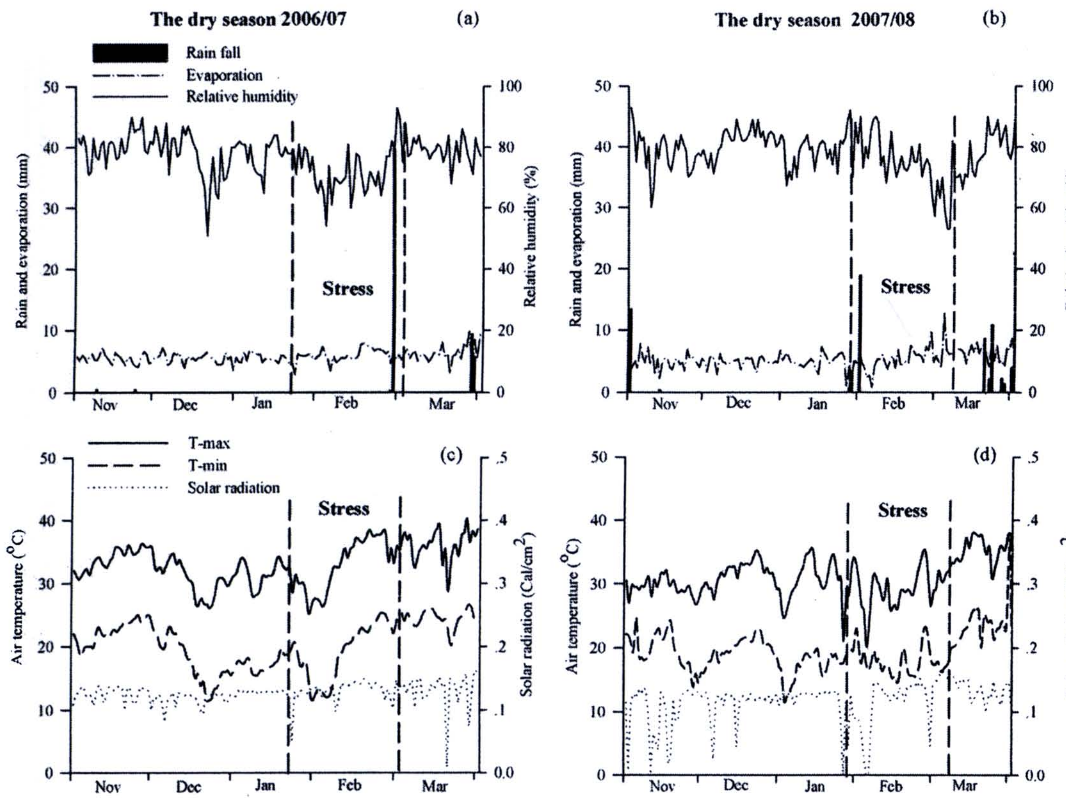

Fig. 1. Relative humidity (%) (a and b), pan evaporation (mm) (a and b), rainfall (mm) (a and b), maximum and minimum air temperature (°C) (c and d), and solar radiation (Cal/cm^2) (c and d) during the crop growth period in 2006/2007 (a and c) and in 2007/2008 (c and d).

2.6. 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 × 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 were 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_E^2) and genotypic variance (δ_G^2), and then broad-sense heritability estimates (h_b^2) were calculated as follows (Holland et al., 2003):

$$h_b^2 = \frac{\delta_G^2}{\delta_P^2} \quad (2)$$

$$\delta_P^2 = \delta_G^2 + \frac{\delta_{GE}^2}{e} + \frac{\delta_E^2}{re}, \quad (3)$$

where h_b^2: broad sense heritability, δ_G^2: genotypic variation, δ_P^2: 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).

3. Results

3.1. 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 (Fig. 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 2006/2007 and 10.2% in 2007/2008, respectively). The soil moisture content of both treatments was held fairly constant from 80 DAP until harvest.

3.2. Variability of parental lines

When all selected parents were tested parallel to 140 progenies, KK 60-3, ICGV 98348, and ICGV 98353 have high SCMR and low SLA under stress conditions, Tainan 9 has high SLA and low SCMR (Table 1). Two drought-resistant lines, ICGV 98348, and ICGV 98353, have low *A. flavus* infection and aflatoxin contamination under stress conditions, while KK 60-3 and Tainan 9 were the otherwise (Table 1).

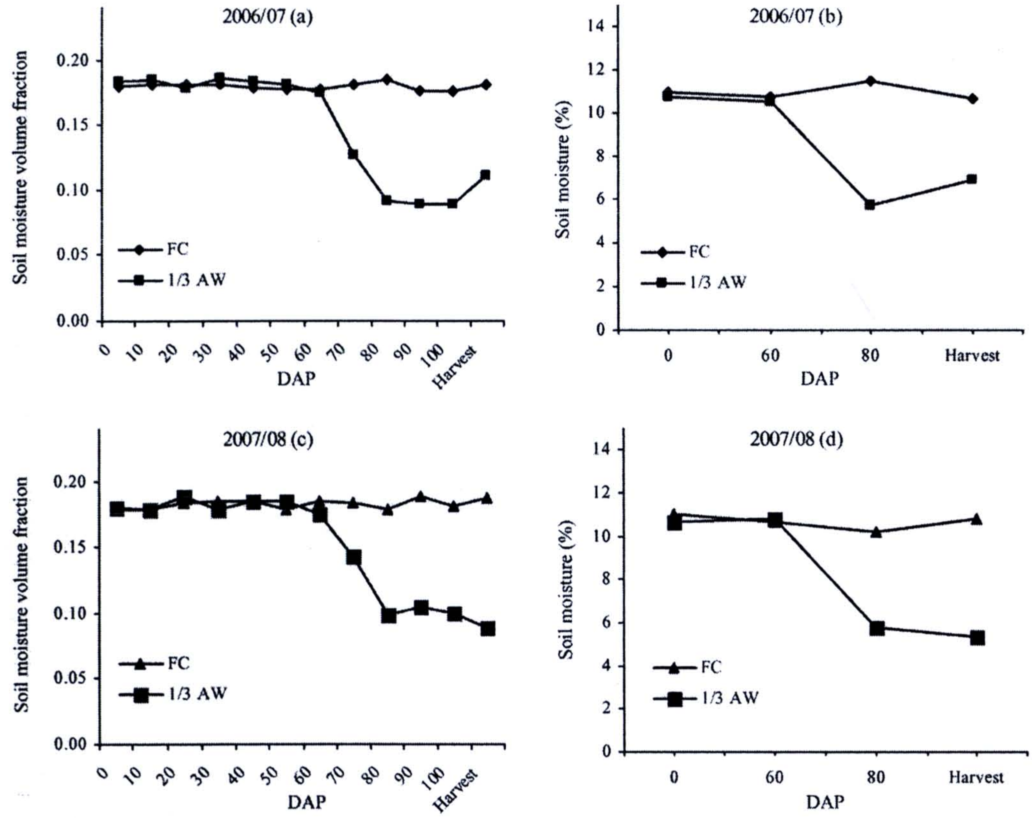

Fig. 2. Soil moisture volume fraction (a and c) at planting, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 days after planting (DAP), and at final harvest and gravimetric soil moisture content (b and d) at planting, 60, 80 DAP and at final harvest under different water regimes [field capacity (FC) and 1/3 available water (1/3 AW)] average from 0 to 60 cm depth in 2006/2007 (a and b) and 2007/2008 (c and d).

3.3. *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 2). 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 × genotypes (Y × G) for seed infection and aflatoxin contamination were also significant ($P \leq 0.05$ to $P \leq 0.01$). Y × G interaction effects for seed infection was higher than for aflatoxin contamination. The significant G × E interaction indicates that aflatoxin traits across environments are inconsistent among genotypes.

3.4. *Yield, physiological traits, and aflatoxin traits under terminal drought*

Wide ranges for pod yield and biomass were observed and reported herein (Table 3). Differences among genotypes for pod yield and total biomass were greater in 2006/2007 than in 2007/2008 (as indicated by the wide ranges of means). Average pod yield in 2007/2008 (2180 kg ha^{-1}) was higher than in 2006/2007

Table 1

Specific leaf area (SLA) and SPAD chlorophyll meter reading (SCMR) at 80, 90, and 100 days after planting (DAP) and *A. flavus* infection and preharvest aflatoxin contamination (PAC) at final harvest of parental peanut under terminal drought in the dry season of 2006/2007 and 2007/2008.

Genotypes	SLA (cm^2 g^{-1})			SCMR			*A. flavus* infection (%)	PAC (ppb)
	80 DAP	90 DAP	100 DAP	80 DAP	90 DAP	100 DAP		
	In the dry season of 2006/2007							
ICGV 98348	147 b	121 c	130 b	42.3 b	46.4 a	50.6 a	23.4 c	544 b
ICGV 98353	131 c	118 c	137 b	44.8 ab	46.9 a	49.4 a	24.6 c	409 b
Tainan 9	174 a	150 a	165 a	36.3 c	36.5 b	40.8 b	36.5 b	1015 a
KK 60-3	148 b	136 b	134 b	47.2 a	46.5 a	50.4 a	45.6 a	904 a
Mean	150	131	142	42.6	44.1	47.8	32.5	718
	In the dry season of 2007/2008							
ICGV 98348	155 b	129 c	120 b	46.0 a	49.1 ab	50.5 a	28.4 b	639 b
ICGV 98353	153 b	148 b	119 b	45.4 a	46.1 b	47.9 a	25.6 b	634 b
Tainan 9	200 a	169 a	144 a	36.4 b	37.7 c	40.9 b	30.1 a	1110 a
KK 60-3	167 b	137 bc	124 b	48.8 a	51.0 a	50.5 a	42.6 a	896 a
Mean	169	146	127	44.2	45.9	47.5	31.7	820

Means in the same column followed by the same letter(s) were not statistically significant by LSD at $P < 0.05$ probability level.

Table 2
Mean squares 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 2006/2007 and 2007/2008.

Source of variation	df	*A. flavus* infection	Aflatoxin contamination
Year (Y)	1	324,212**	7,711,945*
Rep. within Y	6	1256	827,147
Genotypes (G)	139	613**	64,896**
Y × G	139	416**	44,309*
Pooled error	834	412	17,184

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

Table 3
Range and mean 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 2006/2007 and 2007/2008.

Traits	In 2006/2007			In 2007/2008		
	Range	Mean	S.E.	Range	Mean	S.E.
Pod yield (kg h^{-1})	252–4696	2002	37	552–4250	2180	31
Biomass (kg h^{-1})	1825–15,426	7354	102	3302–11,420	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

S.E., standard error for genotypes means.

Table 4
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 2006/2007 and 2007/2008.

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

[a] Standard error.

Table 5
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 2006/2007 and 2007/2008.

Traits	*A. flavus* infection		Aflatoxin contamination	
	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)[a]	−0.07*	−0.11**	−0.23**	−0.57**
DTI (PY)[a]	−0.15**	−0.19**	−0.13**	−0.25**
HI	−0.25**	−0.28**	−0.20**	−0.36**

* and ** are significant at $P<0.05$ and $P<0.01$ level of probability, respectively.
[a] DTI were calculated by the ratio of stressed (1/3 available water (AW))/non-stressed (field capacity (FC)) conditions.

(2002 kg ha^{-1}). In 2006/2007, however, average total biomass was 7354 kg ha^{-1}, and higher than in 2007/2008 (7210 kg ha^{-1}). Mean and range of SCMR were not different between years. Wide ranges of SLA and aflatoxin traits in 2007/2008 were found. In 2007/2008, means of SLA and aflatoxin traits were higher than in 2006/2007 with the exception of SLA at 100 DAP.

3.5. *Heritability of aflatoxin traits*

Heritabilities for seed infection and aflatoxin contamination were low to moderate (Table 4). 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.

3.6. *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 5). 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^{**}).

3.7. *Phenotypic and genotypic correlations between physiological traits and aflatoxin traits*

Close associations between physiological traits for drought resistance and aflatoxin traits were found (Table 6). 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

Table 6
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 2006/2007 and 2007/2008.

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 $P<0.05$ and $P<0.01$ level of probability, respectively.

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.

4. Discussions

Aflatoxin production in peanut appeared to be greatly influenced by the environment. Due to environmental and G × 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, 1996; Holbrook et al., 1994). G × 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. (2010). Utomo et al. (1990) also reported that resistance to seed infection and aflatoxin production in peanut inform the crosses AR-4 × NC 7 and GFA-2 × NC 7 are controlled by difference genes with low heritabilities (ranged from 0.20 to 0.63). Mixon (1976), however, found high heritability estimates for seed infection in a population from the cross PI 337409 × 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., 2009; Girdthai et al., 2010; 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. (2010) 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. (2009) also found that PAC was associated well with SLA, SCMR, root length density, and drought tolerance indices under long period drought conditions. Physiological traits i.e. 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 ($h^2 = 0.81–0.97$) (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 under terminal drought 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 under terminal drought conditions 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.

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Response to Early Drought for Traits Related to Nitrogen Fixation and Their Correlation to Yield and Drought Tolerance Traits in Peanut (*Arachis hypogaea* L.)

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Abstract: This study was aimed to examine the response and contribution of early drought to traits related to N_2-fixation and pod yield and their correlation to drought tolerance. The experiment was conducted at the Field Crop Research Station of Khon Kaen University, Khon Kaen Province, Thailand in the dry season of 2007/08. Eleven peanut genotypes (ICGV 98300, ICGV 98303, ICGV 98305, ICGV 98308, ICGV 98324, KK 60-3, Tainan 9, KKU 72-1, KKU 60, KK 4 and KKU 1) and two soil moisture levels [field capacity (FC) and 1/3 available water (1/3 AW)] were laid out in a split-plot design with four replications. Early drought treatment was given by maintaining 1/3 AW from emergence to 40 days after emergence followed by adequate water supply. The data were recorded for nodule dry weight (NDW) and biomass production (BM) as traits related to N_2-fixation (TNf) at harvest. In addition to, the data on pod yield, number of pod plant^{-1}, number of seed pod^{-1} and seed size (SZ) were also collected at harvest. Specific leaf area (SLA) and SPAD chlorophyll meter reading (SCMR) were measured on 20, 40, 50 and 60 days after emergence (DAE) as drought tolerance traits and harvest index (HI) was calculated after harvest. Early drought did not affect NDW and BM. Major variation was found among peanut genotypes and ICGV 98305 showed higher NDW and pod yield under drought condition. Significant and consistent correlation was found between NDW and BM, ($r = 0.82^*$, $p<0.05$) and ($r = 80^*$, $p<0.05$) under FC and 1/3 AW, respectively. The correlation between TNf and pod yield and yield component parameters varied under the two water regimes. Under 1/3 AW, the only positive correlation observed was between SZ and BM and it might be the only reason for increase in pod yield in some genotypes. SCMR at 60 DAE was strongly related with TNf under both water regimes. There was not any correlation between SLA and HI with NDW and BM. SCMR at 60 DAE is useful to detect chlorophyll density and N_2-fixation under both water regimes because of its high and constant correlation with TNf.

Key words: Peanut, biological nitrogen fixation, SCMR, SLA, water stress, yield components

INTRODUCTION

Peanut is largely grown under rain-fed conditions and its production depends on rainfall and rain distribution that are usually unpredictable (Reddy *et al*., 2003). Droughts occur at any stage of crop development and peanut usually is at the risk of water stress even under irrigated conditions because of limited availability of water and the high cost of energy.

Drought stress, in general, reduces pod yield and other growth parameters of peanut (Pimratch *et al*., 2008b, Songsri *et al*., 2008a). Drought stress during late reproductive phases from pegging to pod filling can greatly reduce pod yield but during pre-flowering growth it has less effect on pod yield or even increases pod yield (Nageswara *et al*., 1985). The mechanisms underlying the ability of peanut to recover from early season drought has not been well researched, especially, in relation to symbiotic N_2-fixation.

Biological N_2-fixation which is crucially beneficial for the development of peanut plants and can support the succeeding crop with the residual fixed nitrogen in the soil is particularly sensitive to adverse environmental conditions such as water stress or drought. Drought affects nodulation, nodule growth and weight as well as nitrogen fixing activity in legumes including peanut (Hungria and Vargas, 2000; Giller, 2001; Pimratch *et al*., 2008a, b). Several traits such as nodule dry weight, biomass production, shoot dry weight, harvest index and leaf color score have been identified and used as selection criteria for high N_2-fixation because the direct ways to measure the fixed nitrogen were too

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costly and laborious (Pimratch *et al.*, 2004; Pimratch *et al.*, 2008b). Pimratch *et al.* (2004) reported that, the leaf color score, nodule dry weight, pod yield and nitrogenase activity were useful indicators for nitrogen fixing ability of peanuts. In general, the effects of drought stress on N_2-fixation and its related traits have been well documented. However, little is known about response of N_2-fixation and its related traits that enhance yield performance when peanut is subjected to early drought stress and further investigations are required.

Earlier investigations demonstrated that several physiological traits could be used for the screening of peanut genotypes for drought resistance. Cruickshank *et al.* (2004) reported three functional components i.e., transpiration (T), transpiration efficiency (TE) and harvest index (HI) as drought tolerance traits. However, specific leaf area (SLA) (leaf area per unit leaf dry weight) has been widely perceived as a trait of drought resistance because it is strongly correlated with TE (Nageswara and Wright, 1994). Further experiments revealed that in peanut, there was significant genetic variability for TE (Wright *et al.*, 1988; Nageswara Rao *et al.*, 1993).

Drought is known to affect chlorophyll content and inhibit the photosynthetic capacity thereafter (Epron and Dreyer, 1993). Samdur *et al.* (2000) and Arunyanark *et al.* (2008) reported that, the leaf chlorophyll content can be rapidly assessed using the SPAD chlorophyll meter reading and it could be used as a rapid and cost effective tool for assessment of relative chlorophyll status in peanut leaves. Moreover, SCMR could be applied for indirect selection of drought tolerance traits in peanut because it is strongly related with SLA (Nageswara Rao *et al.*, 2001) and TE (Sheshshayee *et al.*, 2006).

The improvement of peanut for drought resistance and high N_2-fixation is the main objective of the ongoing peanut breeding program at Khon Kaen University. Pimratch *et al.* (2008a) suggested that maintaining high N_2-fixation under drought stress could be a means for the peanut genotypes to achieve high yield under water limited conditions. Therefore, improvement of N_2-fixation is an alternative strategy to improve pod yield under drought conditions. The information on the responses of peanut genotypes to early drought for N_2-fixation is still lacking and its relationships with pod yield and drought resistance traits are still not well understood. Therefore, the objectives of this study are to observe (1) the response of biological N_2-fixation related traits to early drought and (2) the correlation of the traits related to N_2-fixation to yield and drought tolerance traits in peanut.

MATERIALS AND METHODS

Plant materials: Field experiment was conducted at Khon Kaen University's Field Crop Research Station during December 2007 to April 2008. Eleven peanut genotypes, ICGV 98300, ICGV 98303, ICGV 98305, ICGV 98308, ICGV 98324, KK 60-3, Tainan 9, KKU 72-1, KKU 60, KK 4 and KKU 1 were used in this study. KK 60-3 is a released cultivar commonly grown in Thailand and it is a Virginia-type peanut cultivar with high N_2-fixation (Toomsan *et al.*, 1995) but sensitive to drought for pod yield. Also, KK 72-1 is a Virginia-type peanut cultivar whereas, KK 4 is the only one Valencia-type cultivar. ICGV 98300, ICGV 98303, ICGV 98305, ICGV 98308 and ICGV 98324 are produced from ICRISAT and they are drought resistant Spanish cultivars because they gave high total biomass and pod yield in screening tests under drought stress conditions (Nigam *et al.*, 2005). Tainan 9 is also a Spanish-type peanut cultivar having high SLA, low SCMR under both stressed and nonstressed condition (Songsri *et al.*, 2008a), low dry matter production (Pimratch *et al.*, 2008b) and low N_2-fixation (McDonagh *et al.*, 1993). Also, KKU 1 is a Spanish-type cultivar and a non-nodulating line was used as a reference plant for traits related to N_2-fixation.

Experimental design and treatments: The experiment was arranged in a split-plot design with 4 replications. Where, the main-plot treatments were 2 moisture levels [field capacity (FC) and 1/3 available soil water (1/3 AW)] and sub-plot treatments were 11 genotypes. Plot size was a five rows plot with 3 m long with spacing of 20 cm between plants within row and 40 cm between rows.

Crop management: The land was plowed three times before planting. Lime at the rate of 625 kg ha^{-1} was incorporated into the soil during soil preparation. Phosphorous fertilizer as triple superphosphate at the rate of 24.7 kg P ha^{-1} and potassium fertilizer as muriate of potash (KCl) at the rate of 31.1 kg K ha^{-1} were applied as basal dose before planting. Seeds were treated with a fungicide (Captan) at the rate of 5 g kg^{-1} of seed before planting. Three seeds were planted per hill and thinning was done to obtain one plant per hill at 21 days after planting (DAP). Pre-emergence weed control was carried out by spraying alachlor at the rate of 3 L ha^{-1} after planting and hand weeding was followed at 15 and 35 DAP.

Rhizobium inoculation was done by applying a water-diluted commercial peat-based inoculums of Bradyrhizobium (mixture of strains: THA-201 and THA-205; Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand) on the rows of peanut plants. Gypsum ($CaSO_4$) was applied at the rate of 312 kg ha^{-1} at the pod filling stage. Pest and disease were controlled by weekly applications of carbosulfan at 2.5 L ha^{-1}, methomyl at 1.0 kg ha^{-1} and carboxin at 1.68 kg ha^{-1}. Carbofuran 3% granular was applied at the pod setting stage by side dressing.

Irrigation: Subsurface drip irrigation system was installed to supply water to the peanut plants and soil water level was maintained uniformly at field capacity from planting to 7 DAP in both FC and water stress plots. The soil moisture of water stress plot decreased gradually and from then on it was maintained at the level of 1/3 AW constantly and at ±1% of the pre-determined level until flowering (40 DAE). Water was added to the respective plots by subsurface drip irrigation based on the crop water requirement and surface evaporation which were calculated following the methods described by Songsri *et al.* (2008a).

The calculation of total crop water use for each water treatment was done as the sum of transpiration and soil evaporation. Transpiration (T) was calculated using the methods described by Doorenbos and Pruitt (1992) as follows:

$$\text{ET crop} = \text{ETo} \times \text{Kc}$$

where, ET crop is crop water requirement (mm day^{-1}), Eto is the evapotranspiration of a reference under specified conditions calculated by pan evaporation method and Kc is the crop water requirement coefficient for peanut, which varies with genotypes and growth stage. In addition to, surface evaporation (Es) was calculated with the following formula described by Songsri *et al.* (2008a):

$$\text{Es} = \beta \times (\text{Eo}/t)$$

where, Es is soil evaporation (mm), β is light transmission coefficient measured depending on crop cover, Eo is evaporation from class A pan (mm day^{-1}) and t is days from the last irrigation or rain (day).

Data collection

Weather parameters: The experiment was conducted during the dry season, December 2007 to April 2008. There was maximum rainfall (23.4 mm) at 110 DAE in the dry season (data not shown). The maximum and minimum of seasonal air temperature were 31.7 and 19.7°C, respectively. Daily pan evaporation ranged from 1.78 to 12.68 mm and a seasonal mean solar radiation, 18.0 MJ m^{-2} $days^{-1}$ was observed.

Soil moisture status: Soil moisture in the individual plots was measured by gravimetric soil analysis at planting and harvest at the depths of 0-5, 25-30 and 55-60 cm at 0, 10, 25, 40 and 50 DAP. It could help to calculate the required amount of water to apply. The soil water status was also weekly monitored with a neutron moisture meter (Type I.H. II SER. N° N0152, Ambe Diccot Instruments Co. Ltd., England) at a depth of 0.3 m to 0.6 m at 0.3 m intervals.

Traits related to biological N_2 fixation, yield and yield components: Data were recorded for traits related to N_2-fixation parameters, nodule dry weight and shoot dry weigh at harvest only. Direct N_2-fixation was not determined, because, the earlier investigations have already indicated that, these traits are closely related with N_2-fixation (Pimratch *et al.*, 2004). Plants at the two ends of each row were discarded and only competitive plants were taken as samples. Samples in each plot were dug and separated into parts. Nodules were taken off from the roots and kept separately. Nodules, stems and leaves were oven-dried at 80°C for 48 h and nodule dry weight, shoot dry weight and total dry weight were determined. Pods were air-dried to obtain approximately 8% moisture content and shelled. Then, pod number per plant, seed number per pod, pod yield, seed size and harvest index were determined. To measure the biomass production, bordered plants in an area of 3.36 m^2 were harvested from each plot, then, they were deposed and weighted in the field. A random shoot sample of 2 kg was taken, weighed, then, oven-dried at 75°C for 48 h and dry weight was measured. Shoot dry matter content was calculated and used for determining shoot dry weight for a plot.

Drought tolerance traits: Specific leaf area (SLA) and SPAD chlorophyll meter reading (SCMR) were measured at 20, 40, 50 and 60 days after emergence. The third fully-expanded leaves from their respective terminal bud were detached from 5 chosen plants which were randomly selected from each plot between 8:30 and 9:00 am. SPAD chlorophyll reading was recorded twice on each leaflet of the tetra foliate leaf along the mid-rib. The model of this SPAD chlorophyll meter reading device was Minolta SPAD-502 meter, Tokyo, Japan. Nageswara Rao *et al.* (2001) suggested to fully covering the SPAD meter sensor upon the leaf lamina to avoid the interference from veins and midribs. SLA was measured on these leaves after recording SCMR at the same day. Measuring the leaf area with a leaf area meter (LI 3100C Area Meter, LI-COR Inc., USA) was followed by drying the leaves in an oven at 80°C for at least 48 h and weighted. SLA was calculated using the following formula:

$$\text{SLA} = \text{Leaf area (cm}^2\text{)/Leaf dry weight (g)}$$

Harvest index was computed as the ratio of total pod weight at the final harvest to total biomass at the final harvest. Drought tolerance indexes (DTI) of pod yield (PY) and yield components were calculated using Songsri *et al.* (2008b) formula as follow:

DTI (PY) = Pod yield under stressed treatment/
Pod yield under well-watered condition

Data analysis

Traits related to N_2-fixation and drought tolerance traits: The data were subjected to analysis of variance according to a split plot design. In case of significant difference, mean comparison was done based on Duncan's multiple range test (Gomez and Gomez, 1984). To reveal the comparison between two means of each genotype under two water regimes for nodule dry weight, biomass production and pod yield, the data were separately analyzed with randomized complete block design and least significant difference (LSD) test was used for mean comparison (Gomez and Gomez, 1984). Simple correlations among variables were calculated to determine the relationships among traits (Gomez and Gomez, 1984). Despite collecting SLA and SCMR data on 20, 40, 50 and 60 DAE, the data with the lowest CV and the highest F ratio were considered in data interpretation for precision. SLA data at 50 DAE and SCMR data at 60 DAE were focused to analysis of the variances.

RESULTS AND DISCUSSION

Soil moisture content: Soil moisture was weekly measured using a neutron moisture meter until harvest to directly check whether the water treatments were correct enough or not because water supply was calculated based on weather data. A clear distinction between two soil moisture levels noted at 30 cm of soil depth showed that soil moisture could be controlled along crop development to treat with the desired moisture levels (Fig. 1 a-b). The soil moisture difference due to 2 treatments was smaller at 60 cm depth. Since, there was not any significant change in weather data and no interference of rain during drought treatment, water-regime treatment was carried out correctly until harvest.

Effect of early drought on N_2-fixation related traits: Analysis of variance showed that variety differences were observed for all N_2-fixation traits, nodule dry weight and biomass production but the interaction of variety x water regime (G×W) were not significant for these traits (Table 1). The results indicated that varieties were the most important sources of variation for above traits. Moreover, the differences between two water regimes were not significant for all nitrogen fixing related traits.

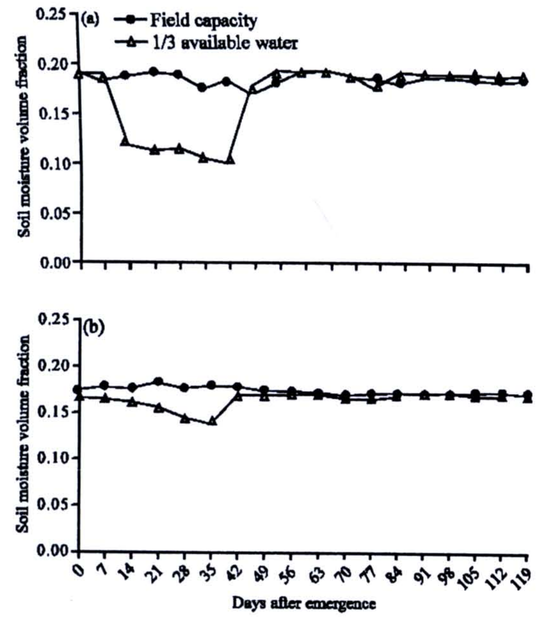

Fig. 1: Soil moisture volume fraction in two available soil water regimes [field capacity (FC) and 1/3 available water (AW)] at 30 cm (a) and 60 cm (b) of the soil level during 2007/08 dry seasons

Perhaps, stressed plants completely recovered from early season drought for N_2-fixation related traits although N_2-fixation decreased under 1/3 AW. On the other hand, since the data of these traits were collected at harvest only, the immediate response of them could not be seen as much as they did. N_2-fixation was affected by early drought because N_2-fixation under irrigated treatment average 350.5 mg N plant^{-1} while the N_2-fixation during recovery period from early drought (40 DAE-final harvest) increased to an averaged of 487.6 mg N plant^{-1} (unpublished data). The analysis also showed that there was no differential responses of genotypes in different moisture regimes (G×W, $p>0.05$) as shown in Table 1. Peanut genotypes showed the different performance in N_2-fixation related traits but they showed uniformly to both water regimes. Stressed plants might response to drought at the end of drought period but each genotype recovered from drought at the harvest time. Since, G×W interaction effects were small, screening and selection of peanut genotypes for high nitrogen fixing ability can be done under all water regimes.

There were differences among the tested genotypes in nodule dry weight at all water regimes ($p<0.01$), ranging from 0.24-0.64 and 0.20-0.68 g plant^{-1} for FC and 1/3 AW, respectively (Table 2). Furthermore, significant differences among tested genotypes were observed in biomass production and it ranged from 5.80-11.05 and

Table 1: Mean square for nodule dry weight, biomass production (BM), pod yield, harvest index (HI), number of pod per plant, number of seed per pod, seed size, specific leaf area (SLA) 50 DAE and SPAD chlorophyll meter reading (SCMR) 60 DAE of 11 peanut genotypes evaluated under two water regimes

SOV	df	Nodule dry wt. (g plant^{-1})	BM (t ha^{-1})	Pod yield (t ha^{-1})	No. of pod plant^{-1}	No. of seed pod^{-1}	Seed size (mm)	SLA 50 DAE (cm^2 g^{-1})	SCMR 60 DAE	HI
Rep.	3	0.006	9.59	0.75	38.35	0.073	109.48	1943.38	9.03	0.001
Water Regime (W)	1	0.003^{ns}	0.09^{ns}	6.66^{ns}	661.12*	0.155**	0.15^{ns}	2425.50^{ns}	71.46*	0.045^{ns}
Error (a)	3	0.022	8.30	2.30	32.65	0.003	28.27	1587.77	6.95	0.02
Genotype (G)	10	0.173**	17.39**	3.73**	98.18**	0.014**	712.27**	2366.00**	56.83**	0.019**
G x W	10	0.014^{ns}	2.23^{ns}	0.75^{ns}	33.06^{ns}	0.015^{ns}	22.32^{ns}	622.74^{ns}	4.28^{ns}	0.012*
Error (b)	60	0.011	2.62	0.48	34.26	0.021	55.57	450.51	5.78	0.006
CV (a)%		31.900	33.69	52.22	25.08	3.120	9.69	19.88	6.01	40.67
CV (b)%		22.600	18.93	23.81	25.69	7.770	13.59	10.59	5.48	21.65

ns: Non significant, *,**Significant at $p<0.05$ and $p<0.01$ levels, respectively. CV: Coefficient of variation, DAE: Day after emergence

Table 2: Nodule dry weight and biomass production of 11 peanut genotypes under different water regimes

	Nodule dry weight (g plant^{-1})				Biomass production (t ha^{-1})			
Genotypes	FC	Significance	1/3 AW	Significance	FC	Significance	1/3 AW	Significance
ICGV 98300	0.59ab	A	0.63a	A	10.40ab	A	9.72abc	A
ICGV 98303	0.59ab	A	0.48ab	A	8.62abc	A	7.40cd	A
ICGV 98305	0.48bc	B	0.58ab	A	8.15bcd	A	8.42bcd	A
ICGV 98308	0.56ab	A	0.40bc	B	7.75bcd	A	8.52bcd	A
ICGV 98324	0.47bc	A	0.56ab	A	10.00ab	A	8.32bcd	A
KK 60-3	0.62a	A	0.66a	A	10.43ab	A	10.52ab	A
Tainan-9	0.36cde	A	0.28c	A	6.47cd	A	6.52d	A
KKU 72-1	0.64a	A	0.68a	A	11.05a	A	11.03a	A
KKU 60	0.39cd	A	0.39bc	A	7.05cd	A	8.57bcd	A
KK 4	0.33de	A	0.26c	A	7.95bcd	A	7.70cd	A
KKU 1	0.24e	A	0.20c	A	5.80d	B	7.62cd	A
Mean	0.48		0.47		8.52		8.57	

Different small letters in each column show significant at $p<0.05$ by Duncan's multiple range test and different capital letters in each row of each parameters show significant at $p<0.05$ by least significant difference test. FC: Field capacity, AW: Available soil water

6.52-11.03 t ha^{-1} for FC and 1/3 AW, respectively (Table 2). The mean performances showed that, KK 60-3 and KKU 72-1 were higher for nodule dry weight and biomass production than other genotypes under both water regimes, whereas, KKU 1 and KK 4 showed the low means for all N_2-fixation parameters under all water regimes (Table 2). These former genotypes can be supposed to be high nitrogen fixing lines because Pimratch *et al.* (2004) reported that high nitrogen fixing lines generally gave high values for weight and number of nodules and shoot dry weight when low nitrogen fixing lines gave low values for these traits.

In addition, the high nitrogen fixing genotypes; KK 60-3 and KKU 72-1 showed the high performance in term of nodule dry weight and biomass production under both water regimes. These consistent genotypes can be effectively evaluated under all water regimes with high N_2-fixation. Another statistical analysis using randomized complete block design and least significant difference (LSD) test revealed that ICGV 98305 showed higher nodule dry weight and pod yield at 1/3 AW, whereas, ICGV 98308 showed lower nodule weight under drought (Table 2).

Effect of early drought on yield and yield components: Statistically, there was a significant variance between two water regimes for number of pod per plant and a highly significant difference in number of seed per pod but the same result was observed for pod yield and seed size (Table 1). Drought tolerance index (DTI) % of all yield and yield components were above one or almost one in most genotypes and it showed that early drought favored the yield of peanut because yield advantages were evident in most genotypes (data not shown). The result agreed with Negeswara Rao *et al.* (1985) who reported that the early drought resulted in a more favorable distribution of dry matter into reproductive components. This increase in yield resulting from decreased irrigation in the early stages of a crop's life may be exploited in crop management when irrigation is available (Negeswara Rao *et al.*, 1985). Analysis of variance showed that there was no interaction of genotypes and water regimes (G×W) in pod yield and yield components (Table 1).

Peanut genotypes did differ for pod yield under both water regimes ($p<0.01$) and they ranged from 1.47-4.37 and 2.12-4.15 t ha^{-1} for FC and 1/3 AW, respectively. Analysis of variance by RCBD and mean comparison with LSD test showed that ICGV 98305 had higher yield (3.00 t ha^{-1})

under water stress than FC (1.90 t ha^{-1}) (Table 3). It seemed to take the advantage of early drought effect because Songsri *et al.* (2008a) reported that percentage of root length density (RLD%) was increased by drought and it was associated with pod yield. Such genotype, ICGV 98305 would be suggested as a parental line for peanut breeding program under drought condition. Higher seed number per pod was observed in KKU 1 under water stress compared to FC (data not shown). Although there was no effect of early drought on pod yield statistically, it affected on other yield components such as number of pod per plant and number of seed per pod and they were increased due to early drought (Table 1).

Effect of early drought on drought tolerance traits: Analysis of variance showed that there was a significant variance in SCMR 60DAE ($p<0.05$) under different water regimes but no effect on HI and SLA 50 DAE (Table 1). This result confirmed the assumption that drought stress reduced chlorophyll content and increased chlorophyll density which causes higher chlorophyll per unit area (Nageswara Rao and Wright, 1994). The differences among genotypes under all water regimes suggested that peanut genotypes were the sources of variation in HI, SCMR and SLA. Five ICGV lines noted as drought resistant lines showed high SCMR values under both water regimes followed by KKU 72-1 (data not shown). Since, DTI of SCMR 60 DAE for all genotypes were more than one except KKU 60 (0.97), it can be clearly seen that early drought increased the chlorophyll density which is vitally important for photosynthesis. For SLA and HI, they were not different under both water regimes but highly significant differences were observed among genotypes. The highest SLA value was found in KK 4 and KKU 1 genotypes under both water regimes. The variation was not observed for drought tolerance traits at the interaction of G x W except for HI. The higher HI was observed in ICGV 98305 under water stress (Table 3). This result totally resembled the data of nodule dry weight and pod yield that also showed better result of this genotype under 1/3 AW (Table 2). It clearly pointed out that all genotypes tried to promote their adaptive ability to early drought as much as they could but ICGV 98305 was significantly resistant to drought and performed so well for most parameters.

Relationship between traits related to N_2-fixation and drought tolerance traits: There was a significant correlation between nodule dry weight and biomass production ($r = 0.82^*$, $p<0.05$) and ($r = 80^*$, $p<0.05$) under FC and 1/3 AW, respectively (Table 4). The result indicated that fixed nitrogen greatly contributed to biomass production rather than pod yield and yield components and the latter parameter can be used as

Table 3: Pod yield and harvest index of 11 peanut genotypes under different water regimes

	Pod yield (t ha^{-1})				HI			
Genotypes	FC	Significance	1/3 AW	Significance	FC	Significance	1/3 AW	Significance
ICGV 98300	2.22def	A	2.85bc	A	0.45a	A	0.30b	A
ICGV 98303	2.40cdef	A	2.95abc	A	0.30bcd	A	0.40ab	A
ICGV 98305	1.90def	B	3.00ab	A	0.22d	B	0.35ab	A
ICGV 98308	2.72cd	A	3.60ab	A	0.37abc	A	0.42ab	A
ICGV 98324	4.37a	A	3.50ab	A	0.32bcd	A	0.42ab	A
KK 60-3	3.20bc	A	3.62ab	A	0.32bcd	A	0.35ab	A
Tainan-9	1.47f	A	2.12c	A	0.22d	A	0.32b	A
KKU 72-1	3.75ab	A	3.57ab	A	0.35bc	A	0.32b	A
KKU 60	2.85bcd	A	4.15a	A	0.40ab	A	0.47a	A
KK 4	2.45cde	A	3.05abc	A	0.32bcd	A	0.37ab	A
KKU 1	1.57ef	A	2.55bc	A	0.27cd	A	0.32b	A
Mean	2.63		3.18		0.33		0.37	

Different small letters in each column show significant at $p<0.05$ by Duncan's multiple range test and different capital letters in each row of each parameters show significant at $p<0.05$ by least significant difference test. FC: Field capacity, AW: Available soil water

Table 4: Correlation coefficients between traits related to N_2- fixation, yield and yield components and drought tolerance traits

Parameter	NDW	BM	PY	No. of pod $plant^{-1}$	No. of seed pod^{-1}	SZ	SLA 50 DAE	SCMR 60 DAE	HI
NDW		0.82*	0.49	0.72*	0.58	0.33	-0.66*	0.63*	0.37
BM	0.80**		0.70*	0.76**	0.65*	0.35	-0.52	0.63*	0.37
PY	0.43	0.58		0.79**	0.65*	0.58	-0.59	0.71*	0.41
No. of pod $plant^{-1}$	0.31	-0.04	-0.01		0.65*	0.58	-0.69*	0.66*	0.53
No. of seed pod^{-1}	-0.09	0.28	0.04	-0.26		0.07	-0.67*	0.36	0.32
SZ	0.44	0.70*	0.78**	-0.42	-0.00		-0.27	0.60*	0.22
SLA 50DAE	-0.53	-0.22	-0.55	-0.41	0.41	-0.23		-0.71*	-0.43
SCMR 60DAE	0.94**	0.71*	0.59	0.26	-0.09	0.49	-0.66*		0.36
HI	-0.15	-0.20	0.67*	0.13	-0.27	0.27	-0.50	0.13	

*,**: Significant at $p<0.05$ and $p<0.01$ levels, respectively. Upper diagonal: Field capacity data and lower diagonal: 1/3 available water data. NDW: Nodule dry weight, BM: Biomass production, PY: Pod yield, HI: Harvest index, SZ: Seed size, SLA: Specific leaf area, SCMR: SPAD chlorophyll meter reading

N_2-fixation related trait in both water regimes because their correlation was consistent. This assumption was more approved because the correlation coefficient between two N_2-fixation related traits, nodule dry weight and BM production and harvest index (HI) was not significant under both water regimes (Table 4).

In contrast, the correlation coefficient between nodule dry weight, BM production and specific leaf area was not significant at 50 DAE under both water regimes. The results might imply that the fixed nitrogen had more partitioning into other vegetative growth than SLA and this is a reason why biomass production is highly correlated with N_2-fixation. The correlation coefficient between nodule dry weight and SCMR 60 DAE was significant (0.63* and 0.94**) at SCMR 60 DAE readings under FC and 1/3 AW, respectively (Table 4). BM production was also highly correlated with SCMR 60 DAE under both water regimes. This result was doubtless because Pimratch *et al.* (2008a) mentioned that N_2-fixation by root nodules depends on the reserve energy supply in the nodules and/or on the photosynthate supply from the shoot. In general, SCMR which is an indirect measurement of chlorophyll density is significantly correlated with biomass production because chlorophyll loss is always associated with reductions in photosynthesis and the large reductions in chlorophyll content might in large part be responsible for the observed reduction in total dry matter.

Relationship between N_2-fixation traits and yield and yield components: Among pod yield and yield components, only number of pod per plant was highly correlated with nodule dry weight (0.72*) under FC (Table 4). Another N_2-fixation related trait, biomass production was highly correlated with pod yield, number of pod per plant and number of seed per pod under FC and it only highly correlated with seed size under 1/3 AW (Table 4). Higher biomass due to fixed nitrogen during water stress could support in pod filling stage after drought treatment. This implies that under drought fixed nitrogen partly supported to vegetative growth rather than to yield. But during the seed filling stage, plant mechanism and N_2-fixation process have already overcame the drought and they restored their ability of contribution to sink thus biomass production was highly correlated with seed size under water stress. It in turn led to higher yield because the correlation between pod yield and seed size was highly significant (0.78**) under water stress (Table 4) and drought tolerance index (DTI) of each genotypes for pod yield was more than one (data not shown).

Relationship between drought tolerance traits and yield and yield components: Correlation coefficient between pod yield and HI was not significant under FC but significant under 1/3 AW (Table 4). The result was beyond question because pod yield is one portion in calculating of HI and HI was sensitive to an interaction effect of G x W (Table 1). Pod yield showed a significant correlation ($p<0.05$) with SCMR 60DAE under FC only (Table 4). The phothosynthate in general provided in pod yield which is one of the sinks but under drought condition they might partially contribute to other growth as fixed nitrogen did. Thus, SCMR 60 DAE was correlated with pod yield under FC only. Number of pod per plant and seed size was significantly correlated with SCMR 60 DAE under FC (Table 4). As a matter of fact, there was a significant effect of early drought on SCMR 60 DAE (Table 1) and higher photosynthesis due to denser chlorophyll might more support to biomass production or nitrogen fixing process than pod yield under early drought. Therefore, there was no correlation between SLA and SCMR and yield and yield components under early drought (Table 4). It can be said that chlorophyll density (SCMR) more determined the photosynthesis to boost the yield compared to the specific leaf area which is one component of chlorophyll content in leaf.

CONCLUSION

The ability of N_2-fixation related traits, nodule dry weight and biomass production were not affected by early drought because peanut plant could recover and undergo in above traits after stress condition. More partition from fixed nitrogen to vegetative growth such as biomass production rather than some yield components might favor bigger seed size with higher yield under water stress.

Water stress did not alter the relationships between N_2-fixation related traits and drought tolerance trait, SCMR. SCMR can be indirectly used as a tool for evaluation of N_2-fixation traits in peanut.

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Inheritance of Traits Related to Biological Nitrogen Fixation and Genotypic Correlation of Traits Related to Nitrogen Fixation, Yield and Drought Tolerance in Peanut (*Arachis hypogaea* L.) Under Early Drought

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Abstract: The improvement of peanut for drought tolerance and high N_2-fixation is the best way to enhance peanut production under drought condition. Besides, the heritability estimates of traits related N_2-fixation and its genetic correlation with yield and drought tolerant traits are useful to formulate the effective breeding program under drought. Therefore, the aims of this study were to estimate the heritabilities (h^2) and genotypic correlation (r_G) among traits related to N_2-fixation (TNf), yield and drought tolerant traits under early drought and non stressed condition. Ninety lines in the $F_{4:8}$ generations from four peanut crosses were tested under Field Capacity (FC) and one-third Available Water (1/3 AW). Data were recorded for Nodules Dry Weight (NDW), Biomass Production (BM), Pods Yield (PY), number of pod plant^{-1}, number of seed pod^{-1} and 100 seed weight at harvest. Specific Leaf Area (SLA), SPAD Chlorophyll Meter Reading (SCMR), Harvest Index (HI) and Drought Tolerance Index (DTI) of PY and BM were measured and calculated as drought tolerant traits. The h^2 for BM, PY, number of pod plant^{-1} and 100 seed weight were high for all tested crosses under both water regimes. With exception of HI trait, high h^2 estimates, also, were found for drought tolerant traits under both water regimes. The genotypic correlation (r_G) between NDW and BM was positive highly significant under both 1/3 AW and FC. BM and PY showed high r_G, whereas, BM and 100 seed weight showed moderate r_G. Moderate r_G was found between BM and SCMR 60 DAE under 1/3 AW and FC. Significant correlations between FC and early drought were found for BM indicating that selection of this trait could be done under both water regimes. BM is possible to select and breed for high N_2-fixation, PY and possibly, drought tolerance because of high h^2 and significant r_G with PY and SCMR 60DAE.

Key words: *Arachis hypogaea* L., N_2-fixation, inheritance, water stress, breeding

INTRODUCTION

Peanut is an important oil seed crop due to its high nutritive value for human diet. Peanut can fix N through the symbiosis process with the help of rhizobium bacteria. This process, partly, supports to accomplish the relatively high yield of peanut and considerable one of the resources of crop's nutrient supply for poor and small-scale farmers who can not effort to use costly chemical fertilizer. Since, peanut yield is dependent on biologically N_2-fixation to a certain extent, improvement of peanut lines for their ability to fix more N may be a suitable approach for peanut yield improvement. Several traits such as nodule dry weight, biomass production, shoot dry weight, harvest index, leaf color score, pod yield and nitrogenase activity have been identified and used as selection criteria for high N_2-fixation and few studies so far have been conducted on the inheritance of these traits (Pimratch *et al*., 2004). High heritability of nitrogenase activity in many leguminous crops such as peanut was reported by Provorov and Tikhonovich (2003) and Sikinarum *et al*. (2007). Arrendrell *et al*. (1985) reported moderate to high heritability estimates for nodule number, nodule and shoot dry weight. Phudenpa *et al*. (2003) observed that, the heritability estimates for all traits related to N_2-fixation were low, especially for leaf color score in which the heritability estimates were zero or near zero in most crosses. Despite the several investigations of genetic variation and heritability for N_2-fixation related traits in peanut breeding population, estimation of heritability for these traits in particular population under irrigated and drought condition would be useful for breeders working with drought. On the other hand, doing a selection among segregated populations under adverse environmental conditions has concurrently brought water stress tolerance. These expected results may provide

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valuable information to monitoring and selecting the high N fixing genotypes under diverse environments.

Like other crops, peanut productivity can be greatly depressed by intermittent drought, which could occur at any time during the growing season when rainfall is inadequate. Also, yield can be reduced by terminal drought, which occurs when stored soil moisture is depleted resulting in crop senescence and reducing pod yield (Subbarao *et al.*, 1995; Serraj *et al.*, 1999). However, there is an interesting result that drought stress during pre-flowering stage can increase yield (Nageswara *et al.*, 1985; Nautiyal *et al.*, 1999). So, exposing peanut plant to pre-flowering drought might be a means to increase peanut productivity.

The selection and breeding of drought tolerant genotypes may help to improve the risky peanut production in drought prone environments. The breeding approach utilizing pod yield has however been unsuccessful, because, it is a quantitative trait and it showed large genotype × environment interactions (Rechards *et al.*, 2001). Recently, other surrogate traits such as Transpiration Efficiency (TE) and Harvest Index (HI) have been used in drought resistance breeding program. Because of their complexity and high cost at the practical work, other easily assessable traits such as Specific Leaf Area (SLA) and SPAD Chlorophyll Meter Reading (SCMR) have become surrogate traits for TE. Samdur *et al.* (2000) and Arunyanark *et al.* (2008) reported that, the leaf chlorophyll density can be rapidly assessed using the SPAD chlorophyll meter reading and it could be used as a rapid and cost effective tool for assessment of relative chlorophyll status in peanut leaves. Since, SCMR is strongly related with SLA (Nageswara *et al.*, 2001) and TE (Sheshshayee *et al.*, 2006), it could be applied for indirect selection of drought resistant traits in peanut. These drought resistant traits such as SLA, SCMR, HI, Drought Tolerance Index (DTI) of biomass DTI (Biomass) and DTI (pod yield) showed high heritability estimates under both non-stressed and stressed conditions (Songsri *et al.*, 2008c). In contrast, Cruickshank *et al.* (2004) reported that broad-sense heritability of transpiration (T), TE and HI were varied among peanut crosses and traits depending on levels of genetic variation in peanut.

There are two possible ways to formulate the development of peanut productivity under drought; (1) improvement of genotypes with high ability of N_2-fixation and (2) breeding of drought resistant genotypes. Both of them are crucially important to obtain the expected high yield. Information of the heritability and the genetic correlations among traits related to N_2-fixation, yield and drought tolerant traits will provide the essential guideline for peanut breeders. Perhaps, drought can alter the inheritance of the interested traits, thus, their heritability should be estimated under different water regimes to clarify the effect of early drought upon the genetic variation and heritability estimates. To our knowledge, no heritability estimates of traits related to N_2-fixation under early season drought and no phenotypic and genotypic correlations for these traits with yield and drought tolerant traits in the literature.

Therefore, the aims of the current study were to estimate the heritability of traits related to N_2-fixation and genotypic correlation among traits related to N_2-fixation, yield and drought tolerant traits under early drought.

MATERIALS AND METHODS

Plant materials: The experiment was conducted at Khon Kaen University's Field Crop Research Station during December 2007 to April 2008. F_1 generations of four peanut crosses (ICGV 98300×KK 60-3, ICGV 98300×Tainan 9, ICGV 98303×Tainan 9 and ICGV 98305×Tainan 9) were generated from the hybridization of 3 drought resistant lines (ICGV 98300, ICGV 98303 and ICGV 98305) selected for low yield reduction with two high yielding cultivars KK 60-3 and Tainan 9. ICGV lines and Tainan 9 have medium seed, whereas, KK 60-3 has large seed and the maturity of ICGV lines, KK 60-3 and Tainan 9 are 110, 120 and 100 days, respectively. KK 60-3 is a released cultivar commonly grown in Thailand. It is a Virginia-type peanut cultivar with high N_2-fixation (Toomsan *et al.*, 1995) but sensitive to drought for pod yield. The F_1 seeds were harvested in bulk for each cross. In F_2 and F_3 generations, two pods were kept for each plant and bulk for each cross. Line separation was carried out in the F_4 generation. A total of 90 lines (25 lines each of first and second crosses and 20 lines each of third and fourth crosses) were randomly selected and they were multiplied in the F_6 and F_7 generations.

The 90 families from 4 crosses were evaluated in the $F_{4:8}$ generations (F_4-derived lines in the F_8 generations, respectively) under two soil moisture levels {field capacity (FC) and 1/3 available soil water (1/3 AW)} in dry season of 2007/08. A split-plot design with four replications was used and plot size was a five rows plot with 3 m long with spacing of 20 cm between plants within row and 40 cm between rows.

Crop management: The land was plowed three times before planting. Lime at the rate of 625 kg ha^{-1} was incorporated into the soil during soil preparation.

Phosphorous fertilizer as triple superphosphate at the rate of 24.7 kg P ha^{-1} and potassium fertilizer as muriate of potash (KCl) at the rate of 31.1 kg K ha^{-1} were applied as basal dose before planting. Seeds were treated with a fungicide (Captan) at the rate of 5 g kg^{-1} of seed before planting. Three seeds were planted per hill and thinning was done to obtain one plant per hill at 21 days after planting (DAP). Pre-emergence weed control was carried out by spraying alachlor at the rate of 3 L ha^{-1} soon after planting and hand weeding was followed at 15 and 35 DAP.

Rhizobium inoculation was done by applying a water-diluted commercial peat-based inoculum of *Bradyrhizobium* (mixture of strains THA 201 and THA 205; Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand) on the rows of peanut plants. Gypsum ($CaSO_4$) was applied at the rate of 312 kg ha^{-1} at the pod filling stage. Pest and disease were controlled by weekly applications of Carbosulfan at 2.5 L ha^{-1}, methomyl at 1.0 kg ha^{-1} and carboxin at 1.68 kg ha^{-1}. Carbofuran 3% granular was applied at the pod setting stage by side dressing.

Irrigation: Subsurface drip irrigation system was installed to supply water to the peanut plants and soil water level was maintained uniformly at field capacity from planting to 7 DAP in both FC and water stressed plots. The soil moisture of water stress plot decreased gradually and from then on it was maintained to lead at the level of 1/3 AW ±1% constantly of the pre-determined level until flowering (40 DAE). Water was added to the respective plots by subsurface drip irrigation based on the crop water requirement and surface evaporation which were calculated following the methods described by Songsri *et al.* (2008a).

The total crop water use for each water treatment was calculated as the sum of transpiration and soil evaporation. Transpiration (T) was calculated using the methods described by Doorenbos and Pruitt (1992) as follows:

$$ET\ crop = ETo \times Kc$$

where, ETcrop: crop water requirement (mm/day), ETo: evapotranspiration of a reference under specified conditions calculated by pan evaporation method, Kc: the crop water requirement coefficient for peanut, which varies with genotypes and growth stage. In addition to, surface evaporation (Es) was calculated using the following formula which described by Singh *et al.* (1993):

$$Es = \beta \times (Eo/t)$$

where, Es is soil evaporation (mm), β is light transmission coefficient measured depending on crop cover, Eo is evaporation from class A pan (mm day^{-1}) and t is days from the last irrigation or rain (day).

DATA COLLECTION

Weather parameters: The experiment was conducted during the dry season, December 2007 to April 2008. The maximum rainfall was 23.4 mm at 110 DAE during experiment established (data not shown). The maximum and minimum air temperature were 31.7 and 19.7°C, respectively. In addition to, daily pan evaporation ranged from 1.78 to 12.68 mm and the solar radiation was 18.0 MJ m^{-2} day^{-1} as a seasonal mean.

Soil moisture status: Soil moisture in the individual plots was measured by gravimetric soil analysis at planting and harvest at the depths of 0-5, 25-30 and 55-60 cm at 0, 10, 25, 40 and 50 DAP to calculate the required amount of water to apply. The soil water status was also weekly monitored with a neutron moisture meter (Type I.H. II SER. N° N0152, Ambe Diccot Instruments Co. Ltd., England) at a depth of 0.3 to 0.6 m at 0.3 m intervals.

Traits related to biological N_2-fixation, yield and yield components: Data were recorded for nodule dry weight and biomass production as N_2-fixation parameters at harvest only. Five plants from each plot were randomly chosen and were carefully dug to recover as many nodules as possible. The plants were cut at ground level to separate roots from shoots. The samples were washed with tap water and nodules were removed from roots by hand. The nodules were oven dried at 80°C for 48 h and the dry nodule was weighed.

Two kilogram random sample of shoots was oven-dried at 80°C for 48 h and dry weight was measured. Pods were air-dried to obtain approximately 8% moisture content and shelled. Then, pod number $plant^{-1}$, seed number pod^{-1}, pod yield, 100 seed weight and harvest index were determined. To estimate the top biomass production with pods, bordered plants in an area of 3.36 m^2 were harvested from each plot, then, the pods were taken off and the roots were cut. The shoots were oven-dried at 75°C for 48 h and dry weight was measured. Biomass production was calculated by combining the dry weight of shoot and pod.

Drought tolerant traits: Specific Leaf Area (SLA) and SPAD Chlorophyll Meter Reading (SCMR) were measured

Table 1: Analysis of variance of cross and cross product

		Mean square of character				
Source of variation	Degree of freedom	X	Y	MCP†	EMS‡	EMCP§
Replication	r-1					
Genotypes (G)	g-1	M_2*	M_2	$M^*_2 M_2$	$\sigma^2_E + r\sigma^2_F$	$\sigma_{E*E} + r\sigma_{F*F}$
Error	(r-1)(g-1)	M_1*	M_1	$M^*_1 M_1$	σ^2_E	σ_{E*E}

†MCP: Mean square of cross product, ‡ EMS: Expected mean square, § EMCP: Expected mean square of cross product

at 20, 40, 50 and 60 days after emergence. The third fully-expanded leaves from their respective terminal bud were detached from 5 chosen plants which were randomly selected from each plot between 8:30 and 9:00 am. SPAD chlorophyll reading was recorded twice on each leaflet of the tetra foliate leaf along the mid-rib. The model of this SPAD chlorophyll meter reading device was Minolta SPAD-502 m, Tokyo, Japan. So, Nageswara *et al.* (2001) suggested to fully covering the SPAD meter sensor upon the leaf lamina to avoid the interference from veins and midribs. SLA was measured on these leaves after recording SCMR at the same day. Measuring the leaf area with a leaf area meter (LI 3100C Area Meter, LI-COR Inc., USA) was followed by drying the leaves in an oven at 80°C for at least 48 h and weighted. SLA was calculated using the following formula:

$$SLA = \text{Leaf area (cm}^2\text{)/Leaf dry weight (g)}$$

Harvest index was computed as the ratio of total pods weight to total biomass at the final harvest. Drought tolerance indexes (DTI) of pod yield (PY) and biomass production were calculated using Songsri *et al.* (2008b) formula as follow;

$$DTI\ (PY) = \frac{\text{Pod yield under stressed treatment}}{\text{Pod yield under well-watered condition}}$$

Data analysis: The data were subjected to analysis of variance according to a split plot design. In case of significant difference, mean comparison was flowed based on Duncan's multiple range test (Gomez and Gomez, 1984). Despite collecting SLA and SCMR data on 20, 40, 50 and 60 DAE, the data with the lowest CV and the highest F ratio were considered in data interpretation for precision. SLA data at 50 DAE and SCMR data at 60 DAE were focused to analysis of the variances.

Estimates of broad-sense heritability for 4 crosses were calculated by the formula described by Holland *et al.* (2003) as follow:

$$h^2 = \sigma^2_G/\sigma^2_P$$

$$h^2 = \sigma^2_G/(\sigma^2_G + \sigma^2/r)$$

Where:

h^2 = Broad-sense heritability
σ^2_G = Genotypic variation
σ^2_P = Phenotypic variation
σ^2 = Error mean square
r = No. of replication

The Standard Error (SE) of heritability (Singh *et al.*, 1993) for traits related to N_2-fixation was calculated to give a measure of the precision of the estimate. Since, the evaluation of heritability estimates was conducted in late generations (F_8) 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 purified through generation advance (Holland *et al.*, 2003).

Phenotypic and genotypic correlation coefficients among traits related to N_2-fixation, pod yield, yield components and drought tolerant traits were calculated based on genotypic means using the following methods which summarized in (Table 1) and described by Falconer and Mackay (1996):

$$\text{Phenotypic correlation } (r_P) = (M^*_2 M_2)/[(M_2^*)(M_2)]^{1/2}$$

$$\text{Genotypic correlation } (r_G) = (M^*_2 M_2 - M^*_1 M_1) / [(M_2^* - M_1^*)(M_2 - M_1)]^{1/2}$$

The value of M_1, M_2, M_1* and M_2* were calculated based on the analysis of variance of cross and cross product (Table 1). Simple correlation was used to determine the relationship between nodule dry weight and biomass production under irrigated and early season drought conditions to understand whether the performance of peanut genotypes was consistent across environments or not.

RESULT AND DISCUSSION

Soil moisture content: Soil moisture was weekly measured using a neutron moisture meter until harvest to directly check whether the water treatments were correct enough or not because water supply was calculated based on weather data. A clear distinction between two soil moisture levels noted at 30 cm of soil depth showed

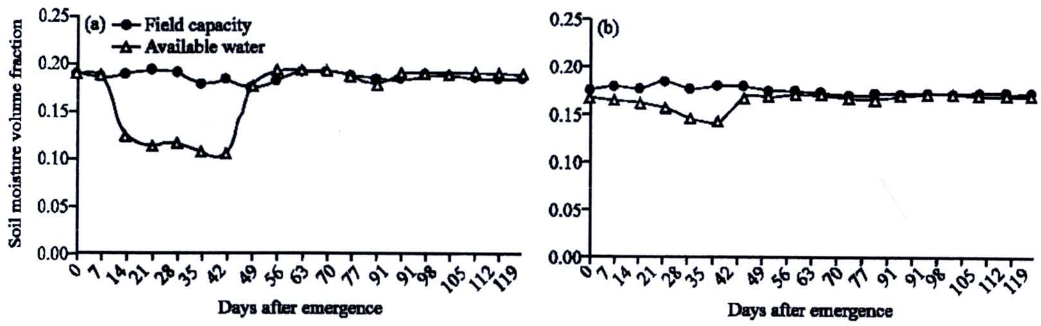

Fig. 1: Soil moisture volume fraction in two available soil water regimes [field capacity (FC) and 1/3 available water (AW)] at 30 (a) and 60 cm (b) of the soil depths during 2007/08 dry season

Table 2: Progenies means for all 4 peanut crosses of 90 lines under early season drought in the dry season of 2007/08

Traits	ICGV 98300×KK 60-3	ICGV 98300×Tainan 9	ICGV 98303×Tainan 9	ICGV 98305×Tainan 9
Traits related to N_2-fixation				
NDW	1.11b	1.20b	1.44a	1.26b
BM	9.71a	7.46b	7.33b	7.66b
Drought tolerance traits				
SLA 50 DAE	166.23b	160.72b	163.41b	177.94a
SCMR 60 DAE	44.99a	44.46a	41.65b	41.82b
HI	0.32ab	0.32ab	0.31b	0.33a
DTI (BM)	1.15ab	1.18ab	1.13b	1.21a
DTI (PY)	1.33b	1.53a	1.24b	1.33b
Yield component traits				
PY	3.98a	2.58b	2.51b	2.61b
No. of pod plant^{-1}	28.68a	26.50b	20.82d	22.45c
No. of seed pod^{-1}	1.89a	1.85a	1.85a	1.88a
100 seed weight	56.21a	37.28d	48.39b	44.57c

Different letter(s) in each row of each parameters show significant at $p<0.05$ by Least Significant Difference test (LSD). NDW: Nodule Dry Weight, BM: Biomass Production, SLA: Specific Leaf Area, SCMR: SPAD Chlorophyll Meter Reading, DAE: Day after Emergence, HI: Harvest Index, DTI: Drought Tolerance Index, PY: Pod Yield

Table 3: Progenies means for all 4 peanut crosses of 90 lines under irrigated condition in the dry season of 2007/08

Traits	ICGV 98300×KK 60-3	ICGV 98300×Tainan 9	ICGV 98303×Tainan 9	ICGV 98305×Tainan 9
Traits related to nitrogen fixation				
NDW	1.13b	1.09b	1.43a	1.34a
BM	8.76a	6.39b	6.65b	6.46b
Drought tolerance traits				
SLA 50 DAE	198.79b	212.30a	197.56b	192.63c
SCMR 60 DAE	42.89a	40.95b	40.04c	39.96c
HI	0.32a	0.28c	0.30b	0.31ab
Yield component traits				
PY	3.24a	1.76c	2.10b	2.05b
No. of pod plant^{-1}	23.40a	22.28a	17.61c	19.84b
No. of seed pod^{-1}	1.88a	1.84a	1.78b	1.85a
100 seed weight	51.54a	36.14c	46.12b	44.30b

Different letter(s) in each row of each parameters show significant at $p<0.05$ by Least Significant Difference test (LSD). NDW: Nodule Dry Weight, BM: Biomass Production, SLA: Specific Leaf Area, SCMR: SPAD Chlorophyll Meter Reading, DAE: Day after Emergence, HI: Harvest Index, DTI: Drought Tolerance Index, PY: Pod Yield

that soil moisture could be controlled along crop development to treat with the desired moisture levels (Fig. 1a-b). The soil moisture difference due to 2 treatments under study was smaller at 60 cm depth. Since, there was not any significant change in the weather data and no interference of rain during drought treatment, water-regime treatment was carried out correctly until harvest.

Mean performances: The mean performances of study traits revealed that ICGV 98303×Tainan 9 cross had higher means for nodules dry weight, whilst, ICGV 983000×KK 60-3 cross had higher means for biomass production under both water regimes (Table 2, 3). In addition to, ICGV 98300×KK 60-3 cross was high in means of most drought tolerant traits such as SCMR 60 DAE, HI and DTI (BM) under early drought. This cross also had the highest means of SCMR 60 DAE and HI under irrigated condition (Table 2, 3). Surprisingly, this cross showed the highest means of pod yield and yield components under both stressed and well-watered conditions. Needless to doubt, the parental line; ICGV 98300 is drought tolerant and high yielding line, while the parental line; KK 60-3 is high in N_2-fixation (Toomsan *et al.*, 1995). On the other hand, ICGV 98303×Tainan 9 cross showed lower means of drought tolerant traits, pod yield and yield components under both stressed and irrigated conditions although it had the high N_2- fixation. Lower means of pod yield were found in ICGV 98300×Tainan 9 and ICGV 98305×Tainan 9 crosses but their means of N_2-fixation related traits were quite high (Table 2, 3).

Heritability of traits related to N_2-fixation, pod yield, yield components and drought tolerant traits: Analysis of variance followed the earlier studies of Arrendrell *et al.* (1985), Pimratch *et al.* (2004) and Sikinarum *et al.* (2007) who reported that genotypes were the main source of significant variation ($p<0.01$) for all N_2-fixation related traits, all agronomic and drought tolerant traits, confirming the presence of variability in the genetic materials (data not shown). Heritability estimates within 4 peanut crosses were calculated for nodule dry weight and biomass production at harvest and shown in Table 4. Heritability estimates for nodule dry weight were low in all crosses under both water regimes and ranged from 0.05 to 0.32. Similarly, Pimratch *et al.* (2004) found low heritability in nodule dry weight (0.00-0.40) of their experimental genotypes, whereas, high heritability estimate for nodule dry weight (0.63-1.00) was reported by Sikinarum *et al.* (2007). The opposite results might be, due to differences in experimental condition and used materials. Present result indicated that, the improvement for these traits will be difficult with least possibility. Besides, heritability of each crosses were consistent under both water regimes showing that early drought could not alter their genetic expression in nodule dry weight. Nigam *et al.* (1985) reported that additive genetic variance was more important than non-additive genetic variance for nodule dry weight. In contrast, Miller *et al.* (1986) reported that, the importance of non-additive genetic variance was greater than additive genetic variance for nodule dry weight. However, selection will be effective, only, when heritability estimate was high and additive genetic effect is greater. Although, ICGV 98303×Tainan 9 cross had low heritability estimates for nodule dry weight, its performance for this trait was high (Table 2). This might be due to high performance of this trait of both parents (high×high). Therefore, improvement for agronomic traits might be possible in this cross if heritability estimates were high.

All crosses showed high heritability estimates for biomass production under both early drought and irrigated conditions as shown in Table 4 and ranged from 0.73 to 0.89. This range was relatively narrow for heritability estimates of biomass and it was a stable factor in this population. Moreover, these similarities between two water regimes showed that, it was possible to select and breed for N_2-fixation using biomass production trait as effective and easy selection tool.

Despite relating to N_2-fixation, generally, the heritability of nodule dry weight was poorer than that of biomass production under two water regimes. In term of practical work, measuring biomass production data is several times easier than that of nodule dry weight. This useful information of high heritability estimates of biomass production and its consistency under early drought and irrigated condition gave a certain extent to achieve the breeding progress for this character.

Table 4: Estimates of heritability with standard error for traits related to N_2-fixation; nodule dry weight (NDW), biomass production (BM) and yield component traits; pod yield (PY), number of pod plant^{-1}, number of seed pod^{-1} and 100 seed weight at harvest of 4 crosses of peanut under Early Season Drought (ESD) and irrigated conditions

	Heritability					
	Traits related to N_2-fixation			Yield component traits		
Crosses	NDW	BM	PY	No. of pod plant^{-1}	No. of seed pod^{-1}	100 seed weight
ESD						
ICGV 98300×KK60-3	0.23±0.05	0.88±0.04	0.84±0.05	0.75±0.07	0.46±0.09	0.87±0.04
ICGV 98300×Tainan 9	0.05±0.02	0.88±0.04	0.58±0.09	0.79±0.06	0.38±0.09	0.93±0.03
ICGV 98303×Tainan 9	0.32±0.07	0.83±0.05	0.80±0.05	0.65±0.08	0.32±0.07	0.93±0.02
ICGV 98305×Tainan 9	0.26±0.11	0.73±0.07	0.74±0.06	0.56±0.08	0.54±0.08	0.94±0.02
Irrigated						
ICGV 98300×KK60-3	0.22±0.06	0.89±0.04	0.86±0.04	0.91±0.03	0.24±0.07	0.79±0.06
ICGV 98300×Tainan 9	0.05±0.02	0.78±0.06	0.88±0.04	0.79±0.06	0.08±0.03	0.87±0.04
ICGV 98303×Tainan 9	0.16±0.33	0.87±0.04	0.85±0.04	0.56±0.08	0.21±0.06	0.85±0.04
ICGV 98305×Tainan 9	0.27±0.11	0.88±0.04	0.88±0.03	0.87±0.04	0.69±0.07	0.93±0.02

ESD: Early Season Drought, NDW: Nodule Dry Weight, BM: Biomass Production, PY: Pod Yield

Table 5: Estimates of heritability with standard error for Specific Leaf Area (SLA) at 50 Days after Emergence (DAE), SPAD Chlorophyll Meter Reading (SCMR) at 60 DAE, Harvest Index (HI) and Drought Tolerance Index of Biomass Production (DTI (BM)) and Pod Yield (DTI (PY)) of 4 crosses of peanut under Early Season Drought (ESD) and irrigated conditions

	Heritability				
	Drought tolerant traits				
Crosses	SLA 50 DAE	SCMR 60DAE	HI	DTI (BM)	DTI (PY)
ESD					
ICGV 98300×KK60-3	0.94±0.02	0.89±0.04	0.19±0.06	0.76±0.07	0.66±0.08
ICGV 98300×Tainan 9	0.87±0.04	0.85±0.05	0.24±0.11	0.71±0.08	0.80±0.06
ICGV 98303×Tainan 9	0.93±0.02	0.83±0.05	0.58±0.08	0.64±0.08	0.67±0.07
ICGV 98305×Tainan 9	0.86±0.04	0.92±0.02	0.07±0.03	0.75±0.06	0.69±0.07
Irrigated					
ICGV 98300×KK60-3	0.75±0.07	0.81±0.06	0.32±0.08	-	-
ICGV 98300×Tainan 9	0.87±0.04	0.80±0.06	0.60±0.09	-	-
ICGV 98303×Tainan 9	0.90±0.03	0.91±0.03	0.70±0.07	-	-
ICGV 98305×Tainan 9	0.85±0.04	0.90±0.03	0.44±0.08	-	-

†DTI were calculated by the ratio of ESD (1/3 Available Water (AW))/non-stressed (Field Capacity (FC)) conditions. ESD: Early Season Drought, SLA: Specific Leaf Area, SCMR: SPAD Chlorophyll Meter Reading, DAE: Day After Emergence, HI: Harvest Index, DTI: Drought Tolerance Index

Most pod yield and yield component traits had similar heritability estimates except number of seed pod^{-1} when they were compared between two water regimes. All crosses showed high heritability estimates for pod yield, number of pod plant^{-1} and 100 seed weight for different water regimes. Heritability estimates for pod yield and number of pod plant^{-1} were moderate to high but widely ranged from 0.58 to 0.88 and 0.56 to 0.91, respectively (Table 4). Also, heritability estimates for 100 seed weight were high and ranged from 0.79 to 0.94, whereas, for number of seed pod^{-1} were moderate in most crosses and quite low (0.08) in ICGV 98300×Tainan 9 cross (Table 4). Present results were not in agreement with Kesmala *et al.* (2004) who reported that estimates of heritability for most agronomic traits were consistently low. Variable results have been reported in other earlier studies concerning to estimation of heritability for agronomic traits in peanut. Nonetheless, the relatively high and moderate heritability estimate for yield and yield component traits in this study were supposed to favor selection in later generations.

Moreover, heritability estimates within four peanut crosses were calculated for HI, SLA 50 DAE, SCMR 60 DAE, DTI (BM) and DTI (PY) (Table 5). HI, DTI (BM) and DTI (PY) were calculated after harvest only. Different heritability estimates were found for HI within four crosses under both water regimes, ranging from 0.07 to 0.70. Variation in HI might be affected by early drought because plant tried to resist water stress by drought tolerant mechanism (Taiz and Zeiger, 2006) and fixed nitrogen might provide to vegetative parts rather than yield under drought (Wunna *et al.*, 2009). HI had different heritability estimates because this trait was a portion of pod yield and it was a quantitative trait with various genetic expressions within peanut crosses. In addition to, other drought tolerant traits such as SLA 50 DAE, SCMR 60 DAE, DTI (BM) and DTI (PY) showed high heritability estimates for four peanut crosses under both water stressed and non-stressed conditions, ranged from 0.64 to 0.94 (Table 5). These results were in agreement with Songsri *et al.* (2008c) who found that estimates of heritability for drought resistant traits were consistently high under both non-stressed and stressed conditions. Early drought could not alter the heritability estimates of such traits.

Genotypic correlation between traits related to N_2-fixation: Phenotypic correlation is not reported because both phenotypic and genotypic correlation showed the same information in this study. Higher genotypic correlation was found between nodule dry weight and biomass production under early drought (0.77, $p \le 0.01$) than irrigated condition (0.65, $p \le 0.01$) (Table 6). Similarly, Pimratch *et al.* (2004) reported that there was high correlation among fixed N, nodule dry weight, shoot dry weight and total dry weight. Fixed nitrogen generally contributed to vegetative growth rather than to yield. Pimratch *et al.* (2008b) reported that correlation between fixed N and biomass production was higher under drought compared to well-watered conditions. The result implied that under early drought peanut nodules might provide fixed nitrogen to nodule development and plant growth. Under drought condition, it was so hard to mine soil nitrogen in drying soil by roots that there was the inability of peanut to use soil nitrogen effectively (Osman *et al.*, 1983) and peanut depended only on biological N_2-fixation. In addition, both traits were measured at harvest only and it reduced the precise with which their genotypic correlation was measured.

Genotypic correlation between traits related to N_2-fixation and yield and yield components: Nodule dry weight had negative and significant correlation with pod

Table 6: Genotypic (r_G) correlation estimates between traits related to N_2-fixation, drought resistant traits and yield component traits for all 4 peanut crosses of 90 lines under Early Season Drought (ESD) and irrigated condition (degree of freedom = 356)

	Traits related to N_2-fixation	
Traits	NDW	BM
ESD		
Traits related to N_2-fixation		
NDW		0.77**
BM	0.77**	
Drought tolerance traits		
SLA 50 DAE	-0.15**	0.25**
SCMR 60 DAE	0.15**	0.57**
HI	0.47**	0.36**
DTI (BM)	-0.15**	0.16**
DTI (PY)	0.10*	0.05
Yield components		
PY	-0.75**	0.79**
No. of pod plant^{-1}	-0.70**	0.47**
No. of seed pod^{-1}	-0.79**	0.41**
100 seed weight	-0.15**	0.52**
Irrigated		
Traits related to nitrogen fixation		
NDW		0.65**
BM	0.65**	
Drought tolerance traits		
SLA 50 DAE	-0.22**	-0.41**
SCMR 60 DAE	-0.07	0.44**
HI	0.01	0.29**
Yield components		
PY	0.10*	0.68**
No. of pod plant^{-1}	0.05	0.44**
No. of seed pod^{-1}	0.09	0.22**
100 seed weight	0.08	0.49**

*and ** significant at $p \le 0.05$ and $p \le 0.01$, respectively. DTI were calculated by the ratio of ESD (1/3 AW)/irrigated (FC) conditions. ESD: Early Season Drought, NDW: Nodule Dry Weight, BM: Biomass Production, SLA: Specific Leaf Area, SCMR: SPAD Chlorophyll Meter Reading, DAE: Day after Emergence, HI: Harvest Index, DTI: Drought Tolerance Index, PY: Pod Yield

yield and yield components under early drought, ranging from (-0.15** to -0.75**) (Table 6). But their correlation was not significant under irrigated condition. Likewise, Pimratch *et al.* (2004) reported that, there was not any correlation among N_2-fixation parameters and agronomic traits except for 100 seed weight. Present results indicated that under early drought, fixed nitrogen might be beneficial to nodules growth and other vegetative parts rather than pod yield.

On the other hand, biomass production was highly correlated with pod yield under early drought and irrigated condition, 0.79** and 0.68**, respectively. The correlation was higher under early drought because fixed nitrogen supplied to plant growth under drought and this advantage contributed to yield after re-watering. Moderate correlation was found between biomass production and number of pod plant^{-1} (0.47**), number of seed pod^{-1} (0.41**) and 100 seed weight (0.52**) under stressed condition (Table 6). Under well-watered condition, the correlation coefficients between biomass production and number of pod plant^{-1}, number of seed pod^{-1} and 100 seed weight were 0.44**, 0.22** and 0.49**, respectively. Between these two traits related to N_2-fixation, biomass production had higher correlation with pod yield and yield components. Progenies means of biomass production and pod yield were generally higher under early drought than well-watered condition (data not shown). These results strongly supported a consumption that fixed nitrogen might be more partitioning to vegetative growth under early drought and the stronger in plant growth in turn contributed to be higher in pod yield. After early drought, peanut plants might need a certain time to recover and then, fixed nitrogen contributed to 100 seed weight by more translocation during seed filling stage. This increase in yield resulting from decreased irrigation in the early stages of a crop's life may be exploited in crop management when irrigation is available (Nageswara *et al.*, 1985). Moreover, these correlations were stable under different water regimes, it would be possible to improve pod yield by selecting of high biomass production in peanut genotypes.

Genotypic correlation between traits related to N_2-fixation and drought tolerant traits: The correlations of nodule dry weight and biomass production with SCMR, SLA and HI were examined in order to better understand these relationships and to assess whether SCMR and SLA could be used as selection tools to enhance N_2-fixation. Nodule dry weight was negatively correlated with SLA at 50 days after emergence (50 DAE) under early drought and irrigated condition, -0.15** and -0.22**, respectively (Table 6). Both N_2-fixation and leaf area were affected by drought (Taiz and Zeiger, 2006) but Sikinarum *et al.* (2007) found that total dry matter accumulation was independent of root nodule or rate of fixation. Therefore, nodule dry weight, otherwise, N_2-fixation was not correlated with SLA because leaf dry weight is a part of SLA. The correlation between nodule dry weight and SCMR 60DAE was low (0.15**) under water stress and negative (-0.07) under irrigated condition (Table 6). As a matter of fact, drought reduced the nitrogen fixation (Hungria and Vargas, 2000; Giller, 2001; Pimratch *et al.*, 2008a, b) and increased the chlorophyll density (Nageswara and Wright, 1994; Arunyanark *et al.*, 2008). The higher chlorophyll density under early drought might provide more photosynthate to other plant growth rather than nodules. This assumption became more obvious with another correlation between nodule dry weight and HI. It was moderate (0.47**) under early drought but very low

Table 7: Correlation coefficients of nodule dry weight (NDW) and biomass (BIO) at harvest of 4 peanut crosses under early season drought (d) and irrigated (w) conditions (degree of freedom (df) = 18 for ICGV 98300×KK 60-3, ICGV 98300×Tainan 9 and df = 23 for ICGV 98303×KK 60-3 and ICGV 98305×KK 60-3)

	Peanut cross			
Correlation	ICGV 98300×KK 60-3	ICGV 98300×Tainan 9	ICGV 98303×Tainan 9	ICGV 98305×Tainan 9
NDW_w vs NDW_d	0.04	0.03	0.13	-0.14**
BIO_w vs BIO_d	0.31**	0.24**	0.37**	0.18**

**Significant at $p \le 0.01$

(0.01) under irrigated condition. Similarly, Pimratch *et al.* (2004) reported that N_2-fixation parameters were negatively correlated with HI under irrigated condition. So, present result indicated that fixed nitrogen contributed to reproductive growth under well-watered condition. Moreover, its correlation with DTI (PY) was quite low (0.10*) whereas its correlation with DTI (BM) was negative (-0.15**) (Table 6).

Furthermore, other N_2-fixation parameter, biomass production was not highly correlated (0.25**) with SLA 50 DAE under early drought and it showed a negative correlation of (-0.41**) under irrigation. Leaf area reduction is the first sign of drought response by plant (Taiz and Zeiger, 2006) but fixed nitrogen might go to biomass production under drought condition. Therefore, their correlations were not highly significant. Nonetheless, its correlation to SCMR 60 DAE was moderate under early drought and irrigated condition, 0.57** and 0.44**, respectively (Table 6). In contrast, high genotypic correlation was reported between biomass production and SCMR under both drought and irrigated condition by Songsri *et al.* (2008c). Therefore, it can be concluded that higher chlorophyll density under early drought might support the biomass production instead of partitioning to sink. Their correlation was so consistent that selection is possible for either fixed nitrogen or drought resistance. Present results did not strongly support the above conclusion, because, their correlation was just moderate. HI was moderately correlated with biomass production under early drought and irrigated condition, 0.36** and 0.29**, respectively (Table 6). It needs not to doubt because as we mentioned before, biomass production was one fraction of HI. But its correlation to other drought tolerant traits such as DTI (BM) and DTI (PY) were quite low and negative (Table 6).

Relationship of traits related to N_2-fixation under early drought versus irrigated condition: Biomass production was possible to be selected under either early drought or irrigated condition, because, biomass production under both water regimes showed a significant correlation in all four peanut crosses (Table 7). Whereas, nodule dry weight showed low correlation values in three crosses of four crosses under study and negative correlation value in remain cross (ICGV 98305×Tainan 9). These correlations of biomass production revealed that more suitable condition for selecting these traits related to N_2-fixation could be done under both well watered and water stressed conditions. A fairly good correlation will favor to select the particular trait under all conditions. In addition, since the heritability estimates of biomass production were high in all peanut crosses under both early drought and irrigated conditions, selection of biomass production is a better way to select high nitrogen fixed genotypes among the tested populations.

CONCLUSION

Peanut lines derived from cross of ICGV 98300×KK 60-3 are recommended because of their high ability to maintain high N_2-fixation, high drought tolerance and high contribution to pod yield under early drought and field capacity. Early drought can not alter the heritability estimates of nodule dry weight and biomass production. It can rather increase the pod yield of some peanut genotypes which can maintain high N_2-fixation under early drought. Based on heritability estimates, biomass production showed a high possibility to improve N_2-fixation among four crosses of peanut population. It may be useful as a selection criterion for high N_2-fixation and pod yield because of, its high genotypic correlation with pod yield and yield components in all 4 crosses. Despite its moderate correlation with SCMR, it would lead to a proposal that SCMR may be an alternative tool of detecting fixed nitrogen to a certain extent in a particular population. Since, measuring nodule dry weight incurs a substantial cost with low heritability and it has low correlation with pod yield and drought tolerant traits, biomass production is possible to select and breed for higher N_2-fixation under early drought and well-watered conditions.

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Heritability and Correlation of Drought Resistance Traits and Agronomic Traits in Peanut (*Arachis hypogaea* L.)

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Abstract: Several physio-morphological traits are related to pod yield of peanut. Improvement of these traits should lead to yield increase under drought conditions. The objective of this study was to evaluate (1) heritability of drought resistance traits, yield and yield components and (2) relationships among these traits. A cross of two parents (ICGV 98324 and KK 4) differing in physio-morphological traits was used in this study. Pot experiments of F_2 and F_3 populations were set up in the open field with rainout shelters. One hundred and twenty eight entries were subjected to water stress during 28 to 70 days after sowing and evaluation of the studied characters was conducted at appropriate time. Data were recorded for Root Dry Weight (RDW), Root Length (RL), Root Surface (RS), Root Volume (RV), Specific Leaf Area (SLA), SPAD Chlorophyll Meter Reading (SCMR), biomass, pod yield, pod number per plant, seed number per pod, 100-seed weight and Harvest Index (HI). Heritability estimates in broad sense for root characters and drought resistance traits were low to intermediate, ranging from 0.27 to 0.59. Similarly, low to intermediate heritability estimates in broad sense were found for pod yield and yield components, ranging from 0.20 to 0.57. Heritability estimates in narrow sense were much lower than in broad sense. The correlation coefficients among root characters were inter-related positively, whereas negative correlation coefficients were observed among physiological characters. Root characters were closely related to biomass production but they were not related to yield and yield components except for pod number per plant.

Key words: Breeding, inheritance, SLA, SPAD chlorophyll meter reading, water stress

INTRODUCTION

Peanut yield in rain-fed areas has been limited by drought stress because pod yield and other growth parameters have been severely affected (Pimratch *et al.*, 2008; Songsri *et al.*, 2008a; Awal and Ikeda, 2002; Nautiyal *et al.*, 2002; Reddy *et al.*, 2003; Nigam *et al.*, 2005). Yield losses have been estimated to be 56-85% (Nageswara Rao *et al.*, 1989), depending on crop growth stages when the crop was exposed to drought (Awal and Ikeda, 2002; Reddy *et al.*, 2003), drought intensity and drought duration (Nautiyal *et al.*, 2002; Nigam *et al.*, 2005). Even in irrigated areas, peanut is frequently exposed to drought because water supply is not sufficient.

The use of drought-resistant varieties is an important strategy to combat the drought problem. These varieties should be able to provide higher yield under drought conditions. Genetic variability for drought resistance has been reported in peanut (Erickson and Ketring, 1985; Upadhyaya, 2005; Songsri *et al.*, 2008a-c, 2009). However, breeding for drought resistance based on pod yield is lacking behind due to significant genotype×environment interactions (Wright *et al.*, 1996).

Alternative breeding strategies using physiological traits as selection criteria have been proposed by some researchers. Rapid progress in drought resistance breeding has been achieved based on characters like Harvest Index (HI), Water Use Efficiency (WUE), Specific Leaf Area (SLA) and SPAD Chlorophyll Meter Reading (SCMR) (Nigam *et al.*, 2005). The SLA and SCMR have been found to be highly correlated with WUE (Nageswara Rao *et al.*, 2001; Sheshshayee *et al.*, 2006) and have been used as surrogate traits for WUE (Nigam *et al.*, 2005; Lal *et al.*, 2006; Sheshshayee *et al.*, 2006; Arunyanark *et al.*, 2008; Jongrungklang *et al.*, 2008; Pimratch *et al.*, 2008). Specific leaf area and SCMR have been found to be negatively correlated (Nageswara Rao *et al.*, 2001; Upadhyaya, 2005).

Earlier studies have indicated differential responses for relative water content in peanut (Painawadee *et al.*, 2009) and it was positively correlated with chlorophyll

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content and grain yield in rice under drought conditions Pirdashti *et al*. (2009). Leaf water status is dependent on rooting density, root distribution, ability of roots to extract water, behavior of stomata closure and transpiration rate (Kramer, 1969, 1983; Gregory, 2006). Root systems play a crucial role in determining shoot water status and therefore effective water uptake is an important determinant of drought resistance (Huang *et al*., 1997; Huang, 2000; Kashiwagi *et al*., 2006). Larger root systems and deep growth of root systems into lower soil profile can take up more water to support plant growth and yield (Ludlow and Muchow, 1990; Turner *et al*., 2001). Moreover, deep and prolific root systems have been associated with enhanced avoidance of terminal drought stress in chickpea (Ludlow and Muchow, 1990; Serraj *et al*., 2004). Selections with more extensive root systems could extract more soil water from greater soil volumes than selections with limited root system. Many root characteristics have been shown to be under genetic control and quantitatively inherited (O'Toole and Bland, 1987). Genetic variation for root characters has been found among peanut genotypes (Ketring, 1984). Differences among peanut genotypes were observed and Virginia type possessed longer taproots and had faster root growth rates than Spanish type (Huang and Ketring, 1987; Maiti *et al*., 2002).

Information on the heritability of RWC, SCMR, SLA, HI, biomass, pod yield, pod number per plant, seed number per pod, 100-seed weight and root traits and the phenotypic correlations among these traits will be useful for planning suitable breeding strategies for improving drought tolerance. The effective selection for traits under improvement depends on sufficient additive genetic variation of the traits that are expressed as heritability. Phenotypic relationships among traits are also important when simultaneous selection of multiple traits is to be carried out for high yield under drought stress conditions. Therefore, the present research was undertaken to estimate the (1) heritability of drought resistance traits, yield and yield components and (2) relationships among these traits.

MATERIALS AND METHODS

Plant materials: Two parental lines (ICGV 98324 and KK 4) were used in a cross to generate an F_1 hybrid. The parents were selected because they were different in many characters such as RWC and SCMR as evaluated in one of our studies (Painawadee *et al*., 2009). ICGV 98324 is a drought resistant line from ICRISAT and it was identified as drought resistant because of high total biomass and pod yield under drought conditions (Nageswara Rao *et al*., 1992; Nigam *et al*., 2003, 2005). KK 4, a released variety in Thailand, is a Valencia bunch type with erect growth habit, early maturity (100 days), small-seeded nature and high drought tolerance index for RDW (Painawadee *et al*., 2009). The F_1 hybrid was further grown in a small plot for multiplication and generation advance. The F_2 and F_3 generations were used in the experiments.

Crop management and experimental designs: The experiment was conducted at the Field Crop Research Station of Khon Kaen University located in Khon Kaen Province, Thailand (latitude 16° 28′ N, longitude 102° 48′ E, 200 m above sea level). One hundred and twenty eight plants in the F_2 generation were first evaluated in an un-replicated trial during December, 2006 to April, 2007 and the 128 progenies in the F_3 generation were later evaluated in a randomized complete block design with four replications during June, 2007 to October, 2007. Crop management for both trials was identical.

The plants were grown in pots with 25 cm diameter and 70 cm height under open environment and removeable rainout shelters were available if necessary. Pots were filled with soil (Yt; fine-loamy, siliceous, isohypothermic, Oxic Paleustults). The soil properties are given in Table 1.

The soil was filled into the pots in four columns of the soil profile in order to make soil bulk density uniform. Three plastic tubes were installed to supply water to each soil column from the bottom to the column below the top soil column and the top soil column was surface irrigated as detailed by Songsri *et al*. (2009) and Painawadee *et al*. (2009).

Lime at the rate of 19.2 g pot^{-1} was incorporated into the soil prior to soil filling and phosphorus fertilizer as triple superphosphate at the rate of 12.12 g P pot^{-1} and potassium fertilizer as muriate of potash (KCl) at the rate 15.26 g K pot^{-1} were applied immediately before planting.

Table 1: Soil properties for F_2 and F_3 generations experiment

	Generations	
Soil properties	F_2	F_3
Physical (%)		
Sand particle	71.00	55.00
Silt particle	20.00	29.00
Clay particle	9.00	16.00
Field capacity	10.00	10.00
1/3 available water	5.00	6.00
Permanent wilting point	3.00	4.00
Chemical		
pH (1:1 H_2O)	5.58	4.50
Organic matter (%)	0.47	0.36
Total nitrogen (%)	0.02	0.03
Available phosphorus (ppm)	7.00	2.00
Potassium (ppm)	23.50	29.50
Calcium (ppm)	216.50	215.50
Bulk density (g cm^{-3})	1.48	1.47

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} seed for fungal control and ethrel 48% at the rate of 2 ml L^{-1} water to break seed dormancy. Rhizobium inoculation was done by applying a water-diluted commercial peat-based inoculum of Bradyrhizobium (mixture of strains THA, 201 and THA, 205; Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand) on the hills before planting.

The seeds were over planted and the seedlings were thinned to one plant per hill at 14 days after sowing (DAS). Gypsum ($CaSO_4$) at the rate of 9.58 g pot^{-1} was applied at 40 DAS. The pots were kept weed free by regular manual weeding. Pests and diseases were controlled by weekly application of carbosulfan [2-3-dihydro-2,2-dimethylbenzofuran-7-yl (dibutylaminothio) methylcarbamate 20% w/v, water soluble concentrate] at 2.5 L ha^{-1}, methomyl [S-methyl-N-((methylcarbamoyl) oxy) thioacetimidate 40% soluble powder] at 1.0 kg ha^{-1} and carboxin [5,6-dihydro-2-methyl-1,4-oxath-ine-3-carboxanilide 75% wettable powder] at 1.68 kg ha^{-1}.

Uniform irrigation at field capacity was supplied to the experiments from planting to 12 DAS. Withholding of irrigation was carried out after 12 DAS to allow soil water gradually get reduced until reaching at 1/3 available water (28 DAS) and the soil moisture content was maintained at this level until 70 DAS. After 70 DAS, soil moisture level at field capacity was brought back until harvest.

The method of calculation for plant water use proposed by Songsri *et al.* (2008a-c, 2009) was followed. It was found that crop water requirement was identical to crop water loss through plant transpiration and soil evaporation. Therefore, crop water requirement is the product of evaporation (a pan) multiplied by crop coefficient for peanut. For each water level, soil moisture was controlled uniformly until harvest.

Weather parameters: Weather data were obtained from the nearest meteorological station. The maximum and minimum air temperatures ranged between 33.1 and 20°C in F_1 trial and 32.4 and 25.8°C in F_2 trial (Fig. 1). Daily pan evaporation ranged from 2.88 to 9.84 mm in F_1 trial and 0.6 to 8.9 mm in F_2 trial. The relative humidity values were 75.6% in F_1 trial and 89% in F_2 trial. The solar radiations and average sunshine hours were 18.9 $MJ/m^2/day$ and 10 h in F_1 trial and 13.9 $MJ/m^2/day$ and 6.4 h in F_2 trial, respectively.

Data collection:

Plant water status: Relative Water Content (RWC) was recorded on each of the four leaflets of the tetrafoliate leaf

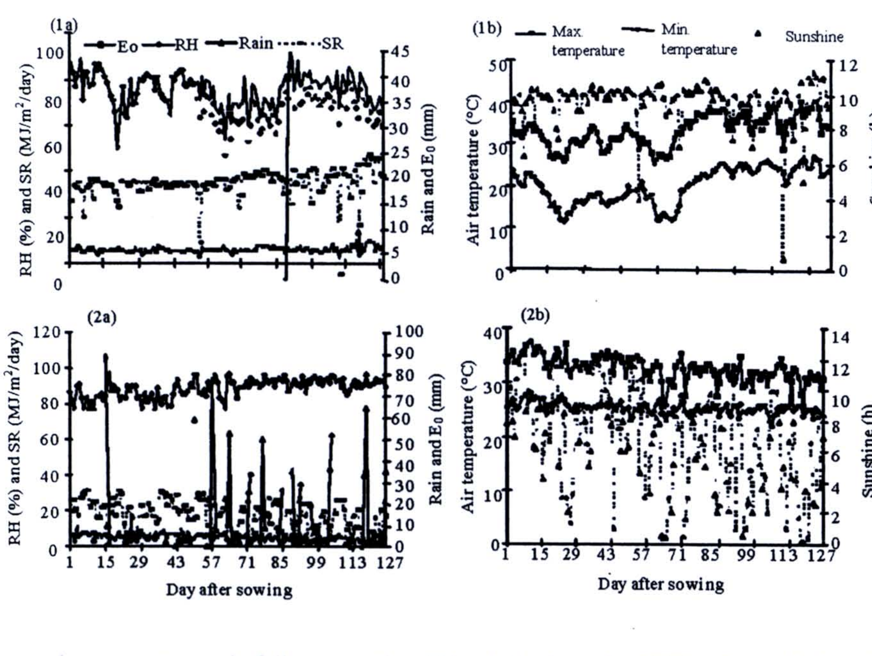

Fig. 1: Daily mean air temperature, rain fall, evaporation (E_o), relative humidity (RH), solar radiation (SR), maximum and minimum air temperature (max. and min. temp.) and sunshine (h) all season grow for experiment 1(1a, b) and experiment 2 (2a, b)

at 70 DAS. Only one fully expanded second or third leaf from the apex of the main axis of each pot was used to record RWC. Leaves were detached and kept in sealable plastic bags in ice box and transported to the laboratory and leaf fresh weight was recorded. The leaf samples were then soaked in distilled water for 8 h and blotted for surface drying and water-saturated leaf weight was determined. The samples were oven-dried at 80°C until reaching constant weight and leaf dry weight could be determined. The RWC was calculated based on the formula suggested by González and González-Vilar (2001) as follows:

$$RWC (\%) = [(FW-DW)/(TW-DW)] \times 100$$

where, FW is the sample fresh weight, TW is the sample turgid weight and DW is the sample dry weight.

Leaf parameters: SPAD Chlorophyll Meter Reading (SCMR), Specific Leaf Area (SLA) and Relative Water Content (RWC) were recoded at 70 days after sowing at 9.00-10.00 AM. The second leaf from terminal bud of the main stem of each plant was detached and kept in sealable plastic bags in ice box. The leaf samples were soon transported to a laboratory. Fresh weight was recorded soon after reaching the laboratory and SCMR was measured immediately by a Minolta handheld portable SCMR meter (SPAD- 502 Minolta, Tokyo, Japan), using four leaflets per sample. In recording the SCMR, care was taken to ensure that the SPAD meter sensor fully covered the leaf lamina and the interference from veins and midribs could be avoided. The same samples were further measured for leaf area, using a leaf area meter (LI 3100C Area meter, LI COR Inc., USA). The samples were then oven-dried at 80°C until reaching constant weight and leaf dry weight could be determined. The SLA was calculated using the following equation (Nageswara Rao *et al.*, 2001):

$$SLA = \frac{\text{Leaf area (cm}^2)}{\text{Leaf dry weight (g)}}$$

Root traits, yield and yield components: Biomass (roots and shoots included), root dry weight, shoot dry weight and harvest index were determined at harvest (120 DAS). Shoots were cut at crown level and the roots were washed in running tap water to remove the soil. The complete removal of the soil was done on a 3 mm sieve. Care was taken to recover the root system completely. The roots were thoroughly cleaned and straightened by repeated dipping and rising in buckets of clear water. Then, The root samples were put on a flatbed scanner (Epson Perfection V700 Photo, Epson Inc., Cheung Shawan, Kowloon, HK) and scanned using WinRhizo Pro 2004a (Regent instruments, Inc., Quebec, QC) at 400 dpi (Gaur *et al.*, 2008; Songsri *et al.*, 2008a). Top and bottom lighting systems were used to eliminate shadows and maximize contrast. The captured grayscale image was analyzed with WinRhizo to measure Root Surface (RS), Root Length (RL) and Root Volume (RV). Root samples and above ground samples were oven dried at 80°C until constant weights were reached. After oven drying, Root Dry Weight (RDW) and shoot dry weight were recorded. Harvest index was calculated using the following relationship (Wright and Nageswara Rao, 1994):

$$HI = \frac{\text{Pod yield}}{\text{Pod yield} + \text{shoot and root dry weight}}$$

At harvest, pods were removed from shoots and immature pods were not included in any calculation of yield parameters. Pod yields were determined after air drying to approximately 8% moisture content. Pod number per plant seed number per pod and 100 seed weight were also recorded at final harvest.

Statistical analysis: The data of the F_3 generation were subjected to analysis of variance (Gomez and Gomez, 1984) and the significant variance of the progenies was further partitioned into genetic variance and error variance. Heritability estimates in broad sense (h^2_{bs}) were calculated using the following relationship (Singh *et al.*, 1993):

$$h^2_{bs} = \sigma^2_g / (\sigma^2_g + \sigma^2_e)$$

Where:
σ^2_e = M_e, σ^2_g = $(M_g - M_e)/b$,
σ^2_e = Environmental variance
M_e = Mean square of error
σ^2_g = Genotypic variance
M_g = Mean square of genotype

The Standard Error (SE) associated with broad sense heritability estimate was calculated as:

$$SE (h^2_{bs}) = (1-h^2)\,[1+(b-1)\,h^2]\,[2/(bf)]^{1/2}$$

Where:
b = Replication
f = Degree of freedom of error.

Heritability estimates in narrow sense (h^2_{rg}) were also calculated by parent offspring method using the data of F_3 on F_2 families as suggested in Smith and Kinman (1965):

$$h^2_{rg} = b/2r_{op}$$

Where:

b = Regression coefficient or slope

r_{op} = Relationship of parents-offspring.

Linear regression coefficients (b) were calculated by regressing of F_3 progeny means (Y_i) on F_2 plants means (X_i). Standard errors (SE) for the slope of each regression were calculated as follows (Ibrahim and Quick, 2001):

$$SE = \left[\left(Y_i^2 - (X_iY_i)2/X_i^2\right)/(n-2)X_i^2\right]/X_i^2$$

where, n is number of families.

Simple correlation coefficients based on progeny means of F_3 generation were calculated to determine the relationship between yield and yield components with drought resistance traits. All calculations were accomplished using STATISTIX 8 software program.

RESULTS

Heritability estimates for root parameters and drought resistant traits: Broad sense heritability estimates for root parameters and drought resistant traits, in general, were much higher than those in narrow sense (Table 2). Heritability estimates in broad sense ranged from 0.27 for SCMR to 0.59 for root surface and root volume, whereas heritability estimates in narrow sense ranged from 0.00 for SLA to 0.13 for root dry weight.

Heritability estimates for yield and yield components: Heritability estimates in broad sense for yield and yield components were relatively higher than those in narrow sense (Table 3). Broad sense heritability estimates ranged from 0.20 for Harvest Index (HI) to 0.57 for pod number per plant, whereas narrow sense heritability estimates ranged from 0.13 for pod yield to 0.23 for pod number per plant and 100-seed weight.

Phenotypic correlation between drought resistant traits and drought resistant traits and yield components: All root parameters (root length, root dry weight, root surface and root volume) were positively and significantly associated with the high correlation coefficients between 0.67, $p \le 0.01$ to 0.98, $p \le 0.01$ (Table 4). In contrast to root parameters, all traits related to drought resistance (RWC, SLA and SCMR) were negatively and significantly associated and the correlation coefficients ranged between -0.17, $p \le 0.05$ to -0.51, $p \le 0.01$). Root characters were also positively and significantly correlated with biomass production and in lesser extent with pod number per plant (Table 5). Most drought resistance traits (RWC, SCMR and SLA) were not correlated with pod yield and yield components except for SCMR with pod number per plant (0.20, $p \le 0.05$).

Table 2: Broad sense and narrow sense heritability and standard errors for root dry weight (RDW), root length (RL), root surface (RS), root volume (RV), specific leaf area (SLA), relative water content (RWC) and SPAD chlorophyll meter reading (SCMR)

	Heritability	
Character	Broad sense	Narrow sense
RDW	0.34±0.03	0.13±0.01
RL	0.58±0.04	0.10±0.00
RS	0.59±0.04	0.11±0.00
RV	0.59±0.03	0.12±0.00
SLA	0.57±0.04	0.00
RWC	0.46±0.04	0.01±0.00
SCMR	0.27±0.03	0.05±0.00

Table 3: Broad sense and narrow sense heritability and standard errors for biomass, pod yield, number pod per plant, number seed per pod, 100-seed weight and harvest index (HI)

	Heritability	
Character	Broad sense	Narrow sense
Biomass	0.30±0.03	0.16±0.00
Pod yield	0.47±0.04	0.13±0.00
Pod No. $plant^{-1}$	0.57±0.04	0.23±0.01
Seed No. pod^{-1}	0.24±0.03	0.19±0.02
100 seed weight	0.46±0.04	0.23±0.01
HI	0.20±0.02	0.21±0.01

Table 4: Correlation coefficients among drought resistance traits for peanut in F_3 generation

	Drought resistance traits					
Drought resistance traits	RDW	RL	RS	RV	RWC	SCMR
RL	0.67**					
RS	0.71**	0.98**				
RV	0.73**	0.92**	0.97**			
RWC	0.03	-0.05	-0.00	0.04		
SCMR	0.15	0.11	0.10	0.09	-0.51**	
SLA	-0.03	0.07	0.06	0.03	-0.17*	-0.40**

*, ** Significant at the $p \le 0.05$ and $p \le 0.01$, respectively

Table 5: Correlation coefficients between yield and yield components with drought resistance traits for peanut in F_3 generation

	Yield and yield components					
Drought resistance traits	HI	Biomass	Pod yield	Pod No. $plant^{-1}$	Seed No. pod^{-1}	100 seed weight
RDW	-0.20*	0.65**	0.13	0.10	0.02	0.04
RL	0.04	0.43**	0.04	0.29**	0.02	0.06
RS	0.00	0.42**	0.01	0.26**	0.02	0.05
RV	-0.04	0.39**	0.04	0.17*	0.01	0.02
SLA	0.02	0.02	0.02	-0.07	0.00	-0.03
RWC	0.04	0.07	0.04	0.11	0.05	0.03
SCMR	-0.01	0.10	0.01	0.20*	0.05	0.00

*, ** Significant at the $p \le 0.05$ and $p \le 0.01$, respectively

DISCUSSION

The progress in breeding for drought resistance in peanut based on pod yield has been slow because of high G×E interactions (Wright *et al.*, 1996). The use of surrogate traits related to drought resistance has been suggested by many authors (Nageswara Rao *et al.*, 2001; Nigam *et al.*, 2005) as the inheritance of these characters must be simpler than pod yield. The information on the heritability and the relationships among characters is important for plant breeders to formulate appropriate breeding strategies to achieve breeding goals. The aims of this study were to understand whether heritability estimates for characters under investigation were sufficient for further improvement of these characters and to explore whether physiological characters and other drought related characters could be used as surrogate traits for pod yield under drought conditions.

For drought resistance and physiological characters, the heritability estimates in broad sense were higher than those in narrow sense. Similar lower narrow sense heritability estimates were also observed for pod yield and its related traits. Higher broad-sense heritability estimates could be due to the inclusion of non-heritable genetic variance and variance due to G×E interactions (Falconer, 1996; Brown and Caligari, 2008). Low heritability estimates observed in this study indicate the difficulty in improving these characters.

The heritability estimates in narrow sense in this study may be under estimated because of high intra plant variation of individual plants in the F_2 generation. Single plants in the F_2 generation might be not appropriate for evaluation of narrow sense heritability estimates and the heritability estimates may be improved if more advanced generations were used with appropriate replicated trials. Root systems themselves are exceedingly complex structures, typically being composed of thousands of individual root axes. Higher narrow-sense heritability estimates for crop growth rate, reproductive duration, partitioning and yield were obtained based on F_4 on F_3 rather than based on F_3 on F_2 (Ntare and Williams, 1998).

Heritability estimates in broad sense and narrow sense for yield and yield components followed a similar pattern to those for root parameters and traits related to drought resistance. However, narrow sense heritability estimates for yield and yield components were somewhat higher than those for root parameters and traits related to drought resistance. Better improvement for these traits would be expected especially for characters with higher narrow sense heritability estimates such as pod number per plant (0.23), seed number per pod (0.19), 100-seed weight (0.23) and harvest index (0.21).

With regard to the heritability estimates, drought resistance and physiological characters were not superior to yield and yield components and therefore, the use of drought resistance and physiological characters as surrogate traits for pod yield and yield components in this population will not be effective. The disappointing results observed in this study could be due to the low genetic variability for these characters in the parental materials. However, the use of more diverse germplasm as parental materials should increase selection efficiency and these surrogate traits are still worth-exploring in peanut.

To the best of our knowledge so far, the report on the heritability for root characters in peanut has not been available in the open literature and the direct comparison of the results is not possible. In peanut, Songsri *et al.* (2008b) found rather high broad sense heritability estimates of 0.73-0.98 for biomass, pod yield, HI, SCMR and SCMR in the $F_{4:7}$ and $F_{4:8}$ generations. For other legumes, however, broad-sense heritability estimates of 0.51-0.55 for root area, 0.47-0.50 for root length and 0.51-0.61 for root mass in common bean under limited soil phosphorus supply has been reported (Araújo *et al.*, 2005). In clover, broad sense heritability estimates for root characters were between 0.2 and 0.4 (Caradus and Woodfield, 1990).

The relationships of root parameters indicated that it was not necessary to evaluate all parameters and evaluation of the most convenient and less expensive characters would be sufficient. In this case, root dry weight is recommended. Ketring (1984) also found positive correlation between root volume and root dry weight in peanut and suggested to select for extensive rooting traits to develop more drought tolerant peanut cultivars. Huang and Ketring (1987) also obtained highly positive linear correlation coefficients for root dry weight with root volume and total dry weight in peanut. High inter-relationships among root characters were also observed in pea (McPhee, 2005) and common bean (Araújo *et al.*, 2005). They also suggested that the high correlation between root mass and root area justifies screening genotypes based solely on root mass. However, negative correlation between root length and root dry weight were found in rice (Sasmal, 2008). This could be due to the fact that rice is normally grown under water-flooded conditions.

In contrast to root parameters, all traits related to drought resistance (RWC, SLA and SCMR) were negatively and significantly associated and the correlation coefficients ranged between -0.17, $p \leq 0.05$ to -0.51, $p \leq 0.01$). The results were in agreement with those reported under non-stressed conditions (Nageswara Rao *et al.*, 2001; Upadhyaya, 2005) and end of season drought conditions (Nigam and Aruna, 2008). More recently, Songsri *et al.* (2009) found consistent relationships between SLA and SCMR under different water regimes. The significant interrelationships between

SLA and SCMR suggested that SCMR could be used as a reliable and rapid measure to identify genotypes with low SLA in peanut. Nageswara Rao *et al.* (2001) reported that SCMR could be used as a reliable and rapid measure to identify genotypes with low SLA or high Specific Leaf Nitrogen (SLN) which are surrogate measures of Transpiration Efficiency (TE) in peanut. In cowpea studied under mid-season drought, the high RWC of leaves was maintained in some of the genotypes by stomata closure and a reduction of leaf area. Drought avoidance by maintaining high leaf water content was negatively associated with SLA (Anyia and Herzog, 2004). Jongrungklang *et al.* (2008) found that the more severe the drought stresses the more was the increase in the SCMR. In fact, plant water status is related to level of soil moisture. Therefore, the results in this study showed the negative and significant relationship between SCMR and RWC. The water loss from cells might effect the concentration of chlorophyll content. During the stress period in rice, SCMR increased with stress but declined rapidly within 3 days after re-irrigation (Duy Nang, 2004). Contrastingly, in rice under drought stress conditions the correlation between SCMR with RWC was positive and significant and SCMR decreased with drought stress compared to control (Pirdashti *et al.*, 2009).

However, drought resistance traits and root traits were not associated. This could be largely due to different times of evaluation between the two groups of characters. RWC, SCMR and SLA were evaluated at 70 DAS, whereas RDW, RS and RV were evaluated at harvest because of limited number of samples and evaluation of root traits being highly destructive. Furthermore, Ketring and Reid (1993) found that groundnut was able to establish both a deep and laterally spreading root system fairly early during the growing cycle, providing adaptation to drought occurrence during and later in the season. Root growth estimations at early vegetative growth stages may be of limited use considering the growth stage×genotype interaction for root growth in chickpea (Canci *et al.*, 2004; Krishnamurithy *et al.*, 1996).

Root characters were also positively and significantly correlated with biomass production and in lesser extent with pods number per plant. This could indicate that larger plants had larger root system and also had high number of pods. Although RDW was not significantly correlated with pod yield and pod number per plant, it was negatively and significantly correlated with harvest index. The results might suggest that the effect of roots contributed indirectly to pod yield through biomass production and subsequently through pod number per plant, but large root systems had low contribution to partitioning efficiency as indicated by negative correlation with harvest index. Similarly, McPhee (2005) observed positive correlation between total root characters and biomass and the present authors also found inter-relationships among root characters. However, Passioura (1983) hypothesized that yield could be increased by decreasing roots as they represent a high energy. Siddique *et al.* (1990) found that wheat genotypes with high HI would have lower root/shoot ratios, indicating less investment in roots. In fact, the turnover of roots can be relatively rapid, with a half life of 30-40 days in peanut (Krauss and Deacon, 1994). Therefore, even if the root/shoot ratio at a given point in time in many species is only between 10 and 40%, a complete turnover of roots in about 40 days would bring the root/shoot ratio close to 100% over the entire life cycle.

Most drought resistance traits (RWC, SCMR and SLA) were not correlated with pod yield and yield components except for SCMR with pod number per plant (0.20, $p \leq 0.05$). More greenish plants yielded more pods than did plants with lighter color under non-stress conditions. According to Wunna *et al.* (2009), number of pod per plant was significantly correlated with SCMR at 60 days after emergence, but the correlation was not significant under 1/3 available water (severe drought stress). SCMR is an indicator of the photo-synthetically active light-transmittance characteristics of the leaf, which is dependent on the unit amount of chlorophyll per unit leaf area (chlorophyll density) (Richardson *et al.*, 2002). Significant and positive correlation between SCMR and chlorophyll content was observed and SCMR was also closely related with chlorophyll density (Arunyanark *et al.*, 2008, 2009).

CONCLUSION

Heritability and correlation information provides a guideline for selection of characters in breeding population. In this study, broad sense heritability estimates for most characters were not high. Narrow sense heritability estimates were much lower than broad sense heritability estimates and they were expected to be under-estimated because of high intra-plant variation in the F_2 population. Based on heritability estimates, selection for RWC, SCMR, root parameters and other traits, characters related to drought resistance and agronomic traits in this population may be difficult in early segregating generation and evaluation should be carried out in more advanced generations. As there were high inter-correlations among root parameters, evaluation of root dry weight alone is sufficient because it was more simple, economical and less time-consuming.

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Identification of Traits Related to Drought Resistance in Peanut (*Arachis hypogaea* L.)

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Abstract: The aim of this study was to investigate whether some root characters and physiological characters are related to drought resistance in some elite germplasm lines earlier identified as drought resistant based on pod yield. Four peanut genotypes were tested in a pot experiment under two soil moisture levels [Field Capacity (FC) and 1/3 available water (1/3 AW)]. A 2×4 factorial experiment was laid out in RCBD with six replications. Data were recorded for Relative Water Content (RWC), Specific Leaf Area (SLA), SPAD Chlorophyll Meter Reading (SCMR), root and biomass at 70 days after planting. Root characters, biomass production, pod yield and Harvest Index (HI) were recorded at harvest and Drought Tolerance Index (DTI) for these traits were also calculated. Differences between water treatments were also significant for RWC, SLA, Root Dry Weight (RDW) and biomass but not significant for SCMR, harvest index and pod yield. Drought stress reduced RWC, SLA, RDW and biomass but had no significant effect on SCMR, harvest index and pod yield. Significant differences among peanut genotypes were found for SLA at both water treatments. ICGV 98353 had the lowest SLA at both water treatments. Peanut genotypes were significantly different for RDW and RWC at 1/3 AW only. KK 4 had the highest RDW. ICGV 98324 performed best for RWC and it also had the highest DTI for RWC. ICGV 98324 also had the highest SCMR, which was significantly different among peanut genotypes at FC.

Key words: Breeding, Groundnut, SLA, SCMR, Water stress

INTRODUCTION

Peanut productivity is often limited by water deficit at certain growth stages during the growing season. Yield losses due to water stress can vary depending on crop growth stages (Awal and Ikeda, 2002; Reddy *et al*., 2003), drought intensity and drought duration (Nautiyal *et al*., 2002; Nigam *et al*., 2005). Although, access to irrigation should eliminate drought problem, it is not possible for most peanut growing areas. Therefore, development of drought resistant varieties, if cannot eliminate, can alleviate the problem.

Attempts have been made at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) to develop drought resistant varieties. Several drought resistant germplasm lines have been identified and released based on high pod yield under drought stress conditions (Nageswara Rao *et al*., 1992; Nigam *et al*., 2003, 2005). These germplasm lines are valuable as germplasm sources to transfer drought resistance traits to high yielding well-adapted cultivars. However, selection for pod yield is difficult because of high genotype × environment interaction (G×E).

More simple and effective selection schemes have been explored using surrogate traits for drought resistance. Specific Leaf Area (SLA), SPAD Chlorophyll Meter Reading (SCMR), Water Use Efficiency (WUE), Harvest Index (HI), biomass production and Drought Resistance Index (DTI) have been used as surrogate traits for drought resistance in peanut (Nigam *et al*., 2005; Arunyanark *et al*., 2008; Jongrungklang *et al*., 2008; Pimratch *et al*., 2008). More rapid progress may be achieved by using physiological traits such as HI, WUE, SLA and SCMR (Nigam *et al*., 2005). SLA and SCMR have been used as surrogate traits for WUE (Wright *et al*., 1994; Nageswara Rao and Wright, 1994; Sheshshayee *et al*., 2006; Nigam *et al*., 2005). Water Use Efficiency was associated with SCMR, SLA and carbon isotope discrimination ($\Delta^{13}C$) (Lal *et al*., 2006) and Transpiration Efficiency (TE) was also associated with SCMR, SLA and carbon isotope discrimination (Krishnamurthy *et al*., 2007). Nageswara Rao and Wright (1994) found that associations of SCMR and SLA were relatively stable across environments. Leaf photosynthesis is generally correlated with chlorophyll content per unit leaf area and SPAD chlorophyll

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meter can provide a useful tool to screen for genotypic variation in potential photosynthetic capacity (Nageswara Rao *et al.*, 2001).

Root characters are also important for breeding for drought resistance as roots can extract water from the soil (Wright and Nageswara Rao, 1994). Deep rooting and root length density have been identified as drought-adaptive traits that can be used as selection criteria for drought resistance traits (Ludlow and Muchow, 1990; Matsui and Singh, 2003; Taiz and Seiger, 2006). Rucker *et al.* (1995) found that some peanut genotypes with large root systems under non-stress conditions gave high yield under drought conditions. Drought stress generally reduces root growth rate (Meisner and Karnok, 1992). However, Songsri *et al.* (2008c) reported that drought stress increased Root Length Density (RLD) of peanut in the deeper subsoil layers.

It would be expected that drought resistant peanut genotypes previously identified by ICRISAT might possess or be superior for some surrogate traits for drought resistance. They might perform well for root characters under drought conditions that support them to take up more water. They might retain high water content (Relative Water Content; RWC) in their leaf tissues or have greater photosynthesis apparatus (Specific Leaf Area; SLA and traits related to chlorophyll content) to support high photosynthesis under drought conditions. Identification of these drought resistance traits should facilitate selection schemes for drought resistance. Unfortunately, this useful information has not been available in the literature and further investigations are necessary. The objectives of this study was to investigate whether some root characters and physiological characters are related to drought resistance in some elite germplasm lines earlier identified as drought resistant based on pod yield.

MATERIALS AND METHODS

Experimental conditions and materials: The pot experiment was conducted under open environment in the field at the Field Crop Research Station of Khon Kaen University located in Khon Kaen Province, Thailand (latitude 16° 28' N, longitude 102° 48' E, 200 m above sea level) during November 2006 to April 2007. Rainout shelters were available if necessary. Soil type is Yasothon Series (loamy sand, Ocix Paleustults) with the following soil chemical attributes: pH of 5.50-5.65, poor in organic matter (0.43-0.51%), total nitrogen (N) (0.02-0.03%), available phosphorus (P) (6.0-8.0 ppm), potassium (K) and calcium (Ca) (23.5 and 216.5 ppm, respectively).

Three peanut lines (ICGV 98303, ICGV 98305 and ICGV 98324) kindly donated from ICRISAT and a cultivar

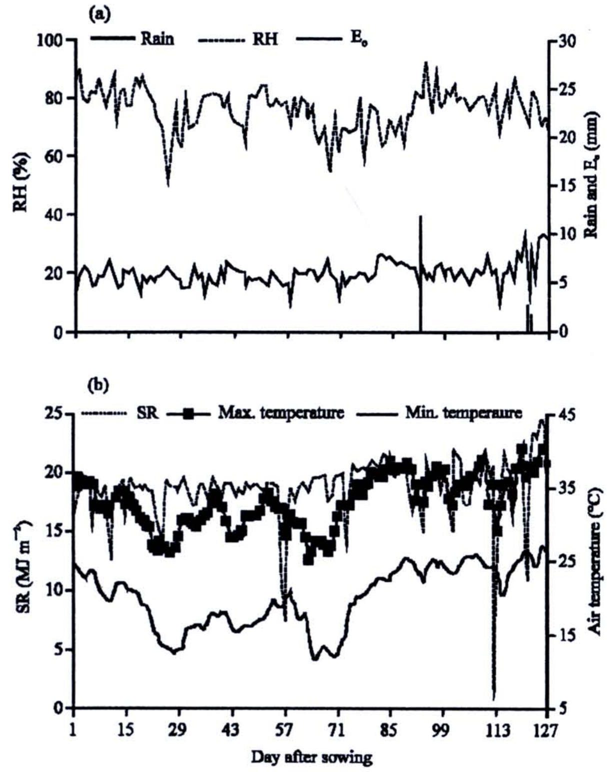

Fig. 1: Rain fall, evaporation (E_o), Relative Humidity (RH) (a), maximum and minimum air temperature (max. and min. temp.) and Solar Radiation (SR) and (b) all season grow

(KK 4) released in Thailand were used in this study. The lines from ICRISAT were identified as drought resistant because they produced high total biomass and pod yield in screening tests under drought conditions (Nageswara Rao *et al.*, 1992; Nigam *et al.*, 2003, 2005) The experimental design was a 2×4 factorial in RCBD with six replications. Two soil moisture levels FC (10.28%) and 1/3 AW (5.33%) were assigned as factor A and four peanut genotypes were assigned a0s factor B. Weather data were obtained from a meteorological station just 50 m from the experimental site and are shown in Fig. 1.

Crop management: Pots with 25 cm in diameter and 70 cm in height were filled with 43.6 kg of dry soil from bottom to 10 cm below the top to create uniform bulk density. As soil pH was low and major nutrients were insufficient, lime at the rate of 19.2 g pot^{-1} was incorporated into the soil prior to soil filling and phosphorus fertilizer as triple superphosphate at the rate of 12.12 g P pot^{-1} and potassium fertilizer as muriate of potash (KCl) at the rate 15.26 g K pot^{-1} were applied soon 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} seed before planting also treated with ethrel 48% at the rate of 2 ml L^{-1} water to break dormancy. Rhizobium inoculation was done by applying a water-diluted commercial peat-based inoculum of Bradyrhizobium (mixture of strains THA 201 and THA 205; Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand) on the holes before planting. Three to four seeds were planted per hill and the seedlings were thinned to two plants per hill at 14 Days After Sowing (DAS). Gypsum ($CaSO_4$) at the rate of 9.58 g pot^{-1} was applied at 40 DAS. Weeds were controlled by hands during the remainder of the season. 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 2.5 L ha^{-1}, methomyl [S-methyl-N-((methylcarbamoyl) oxy) thioacetimidate 40% soluble powder] at 1.0 kg ha^{-1} and carboxin [5,6-dihydro-2-methyl-1,4-oxath-ine-3-carboxanilide 75% wettable powder] at 1.68 kg ha^{-1}.

Initial soil moisture for all water treatments was maintained at field capacity (10.28%) from planting to 14 DAS. Withholding water was initiated for stressed treatment after 30 DAS and the soil moisture were kept constant at 1/3 AW (5.33%) until 70 DAS and then was resumed at FC until harvest, whereas the non-stressed treatment was kept at FC until harvest.

The calculation for plant water use was followed the method described by Songsri *et al.* (2008a-c). Briefly, crop water requirement was identical to crop water loss through plant transpiration and soil evaporation, ignoring other losses. Therefore, crop water requirement is the product of evaporation (a pan) by crop coefficient, which depends on crop species and growth stages.

For each water treatment, soil moisture was maintained as a constant level as possible, allowing no less than 1% variation. In maintaining the defined soil moisture contents, water was filled into the pots at calculated amount at the top of the pots by surface irrigation and three levels of the soil profile through plastic tubes. This irrigation method provided uniform soil moisture to the pots.

Data collection

Weather parameters: There were three rainfalls occurring during crop growth period (Fig. 1). The first rain of 39 mm occurred at 95 DAS and the second and third rains of 9.4 and 5.7 mm occurred at 124, 125 DAS, respectively. As rainout shelters could adequately protect the crop, rainfalls did not have significant effects on the crop. The seasonal mean maximum and minimum air temperature ranged between 20.1 and 33.2°C. Low temperature was observed during 24-34 and 64-72 DAS. Daily pan evaporation ranged from 2.9 to 9.84 mm. The seasonal mean solar radiation 18.79 MJ m^{-2} day^{-1} in 2006-07, were observed.

Soil moisture and plant water status: Soil moisture was determined by gravimetric soil analysis at 50, 60 and 70 DAS. Gravimetric soil analysis at planting was conducted to calculate correct amount of water applied to the crop for successive irrigations. Simultaneously, Relative Water Content (RWC) was recorded from four leaflets of the second fully expanded leaf from the top of the main stem for each pot. Once leaves were harvested and transported to the laboratory, leaf fresh weight was recorded. The leaf samples were then soaked in distilled water for 8 h and blotted for surface drying and water-saturated leaf weight was determined. The samples were oven-dried at 80°C until reaching constant weight and leaf dry weight could be determined. RWC was calculated based on the formula suggested by González and Gonzanlez-Vilar (2001) as follows:

$$RWC\ (\%) = \frac{(FW - DW)}{(TW - DW)} \times 100$$

where, FW is the sample fresh weight, TW is the sample turgid weight and DW is the sample dry weight.

Leaf parameters: SPAD Chlorophyll Meter Reading (SCMR) and Specific Leaf Area (SLA) were recorded at 70 days after sowing at 9.00-9.20 AM. The second leaf from terminal bud of the main stem of each plant was detached and kept in sealable plastic bag in ice box. The leaf samples were soon transported to a laboratory. Fresh weight was recorded soon after reaching the laboratory and SCMR was measured immediately by a Minolta handheld portable SCMR meter (SPAD-502 Minolta, Tokyo, Japan), using four leaflets for a sample.

The same samples were further measured for leaf area, using a leaf area meter (LI 3100C Area meter, LI COR Inc., USA). The leaf samples were then oven-dried at 80°C until reaching constant weight and leaf dry weight could be determined. SLA was calculated as following equation;

$$SLA = \frac{\text{Leaf area (cm}^2\text{)}}{\text{Leaf dry weight (g)}}$$

Biomass, root and Harvest Index (HI): Because of limited samples, biomass, root and HI can be determined at

70 DAS and additional pod yield in final harvest. Plants were cut at crown level. Roots were washed in tab water to remove soil from the roots. Care was taken to recover all roots as many as possible. Root surface, root length and root volume were then determined by WINRHIZO Pro 2004a software. Root samples and above ground samples were oven-dried at 80°C for 48 h. After oven-dry, root dry weight and shoot dry weight were determined.

Drought Tolerance Index (DTI) was calculated for pod yield as suggested by Nautiyal *et al.* (2002) using the relationship as follows:

$$\text{DTI(PY)} = \frac{\text{Pod yield under stressed conditions}}{\text{Pod yield under non-stressed conditions}}$$

DTI is a ratio of the trait evaluated under drought conditions and under fully-irrigated conditions. Therefore, High DTI values indicate drought resistance and vise visa. Other DTIs were also calculated for RWC, SLA, SCMR, harvest index and biomass production.

Harvest index was also calculated using the following relationship:

$$\text{HI} = \frac{\text{Pod yield}}{\text{Pod yield} + \text{shoot and root dry weight}}$$

Statistical analysis: The data were subjected to analysis of variance followed a factorial experiment in a 2×4 RCBD and Duncan's multiple range test was used to compare means (Gomez and Gomez, 1984). As there were interactions between water regime and peanut genotypes, the separated analyses of each water regime were reported.

RESULTS AND DISCUSSION

Soil water status showed reasonable management of soil moistures (Fig. 2). A clear distinction among soil moisture levels was noted at 50, 60 and 70 DAS. Soil with full irrigation therefore, the difference in RWC between fully-irrigated plants and stressed plants at 70 DAS was similar to the difference in soil moisture content. Plants could maintain leaf turgor under drought stress, but, under severe drought stress, leaf turgor was rapidly lost (Reddy *et al.*, 2003). Katam *et al.* (2007) suggested that plants respond to adopt the stress for survival through homeostasis or osmotic adjustment involving changes in physiological and biochemical processes. During the stress, plants may maintain water uptake via osmotic adjustment, which lowers the water potential of the leaf and maintains an osmotic gradient in the leaves.

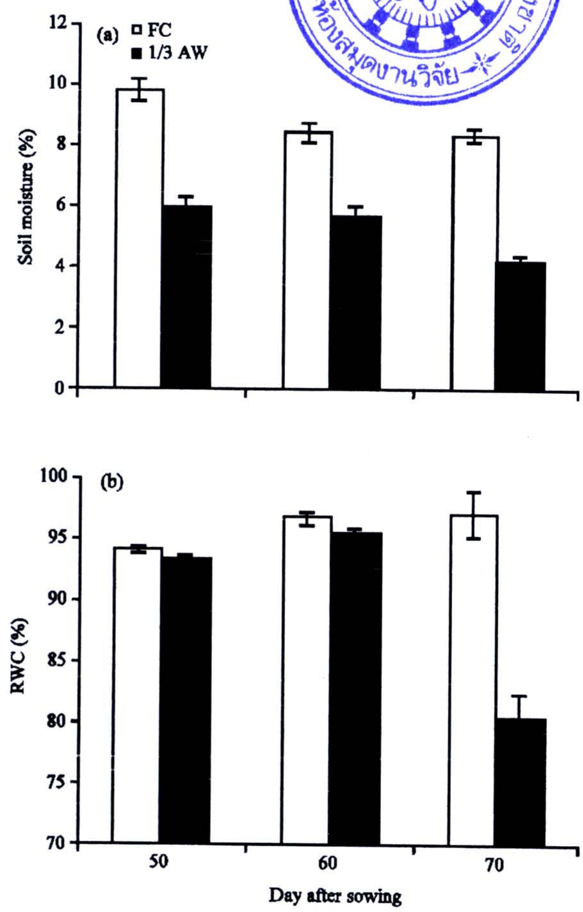

Fig. 2: Soil moisture (a) and Relative Water Content (RWC) (b) under field capacity and 1/3 available water (1/3 AW) at 50, 60 and 70 day after sowing

Ericson and Ketring (1985) reported substantial osmotic adjustment peanut, ranging from 0.6-0.9 MPa. There was also evidence that there were significant cultivar differences in the extent of adjustment. Stirling *et al.* (1989) showed that, while substantial osmotic adjustment between 0.84-1.58 MPa occurred in expanding leaves. This response allowed expanding leaves to maintain higher turgor levels during periods of stress. The situation regarding the importance, extent and possible cultivar variation in osmotic adjustment in groundnut is therefore unclear.

Identifying peanut genotypes with drought resistance: The materials used in this study are peanut genotypes previously identified as drought resistant based on pod yield and biomass production under drought conditions (Nageswara Rao *et al.*, 1992; Nigam *et al.*, 2003, 2005). They were tested in pot experiment using KK 4, a well-adapted high yielding cultivar, as a drought susceptible

Table 1: Biomass, pod yield and Drought Tolerance Index (DTI) for four peanut genotypes under Field Capacity (FC) and available water (1/3 AW) condition at harvest

	Biomass (g plant^{-1})								
	70 DAS			At harvest			Pod yield (g plant^{-1})		
Genotypes	FC	1/3 AW	DTI	FC	1/3 AW	DTI	FC	1/3 AW	DTI
ICGV 98303	9.0	7.6	0.88	16.8	15.5	0.93	5.79	4.59	0.81
ICGV 98324	11.8	7.7	0.65	15.2	13.6	0.91	6.26	5.14	0.93
ICGV 98353	10.2	6.5	0.65	14.7	13.4	0.92	4.72	5.45	1.17
KK 4	12.5	8.7	0.70	12.6	13.3	1.06	4.71	4.54	0.96
Mean	10.9A	7.6B	0.72	14.8	14.0	1.00	5.37	4.93	0.97

For water regime comparison, Mean values in the same row with the same capital latter(s) were not significantly different by LSD at $p \le 0.05$ DTI for genotype were calculated by the ratio of stressed (1/3 AW)/non stress (FC) conditions

Table 2: Harvest index (HI), SPAD Chlorophyll Meter Reading (SCMR) and Drought Tolerance Index (DTI) for four peanut genotypes under Field Capacity (FC) and available water (1/3 AW) conditions at harvest

	HI			SCMR		
Genotypes	FC	1/3 AW	DTI	FC	1/3 AW	DTI
ICGV 98303	0.19	0.18	0.91	40.8b	39.8	0.97
ICGV 98324	0.21	0.21	1.07	46.1a	44.7	0.98
ICGV 98353	0.18	0.22	1.22	41.5b	42.7	1.03
KK 4	0.20	0.19	1.01	38.9b	45.7	1.19
Mean	0.20	0.20	1.05	41.8	43.2	1.04

For comparison among peanut genotypes, Mean values in the same column with the same letter(s) were not significantly different by LSD at $p \le 0.05$. DTI for genotype were calculated by the ratio of stressed (1/3 AW)/non stress (FC) conditions

check. Drought stress significantly reduced biomass production from 10.9-7.6 g plant^{-1} (Table 1). Drought stress seemed to reduce pod yield from 5.4 to 4.9 g plant^{-1}, but the reduction was not statistically significant. Drought stress had no significant effect on harvest index. Drought Tolerance Indices (DTI) were not statistically significant for biomass production, pod yield (Table 1) and HI (Table 2). The expectation is that the ICGV genotypes should be better than KK 4 for biomass production and pod yield at least under drought conditions, but they performed similar to KK 4 for these traits. The results led to conclusion that KK 4 was tolerant to drought similar to the ICGV genotypes (based on pod yield and biomass production). This could be due to the fact that, although KK 4 has not been tested for drought resistance, it has been grown widely under rain-fed conditions in Thailand and it showed good stability for pod yield.

There was no significant genotypic difference in biomass production, HI, pod yield and DTI at any water level. This could be due to low variation for these characters. Another possible reason is that peanut genotypes responded in a similar pattern. For example, the recovery for biomass did not exceed its potential in any peanut genotypes, but full recovery was found for HI and pod yield, making no significant difference between water regimes. RWC could be recovered as early as 24 h after re-watering in line with the recovery of leaf stomatal conductance, the electron transport rate and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) activity (Lauriano *et al*., 2004). Awal and Ikeda (2002) also found the relief from deficit that enabled the plants to reconstitute greater amounts of chlorophyll pigments and to regain foliar water status and stomatal conductivity simultaneously, resulting in higher amounts of gas exchange, especially through photosynthesis and thus increased water use efficiency and quantum yield. Lauriano *et al*. (1997) reported that photosystem I (PSI) and PSII activities in peanut were much higher after recovery than in controls. The capacity for rapid recovery after moisture deficit indicates that peanut has a greater ecophysiological plasticity than other crop plants, enabling it to grow well in drought-prone environments (Awal and Ikeda, 2002). Peanut plants also have a drought recovery mechanism operating at a late growth stage, but might not maintain a source-sink balance and so the yield loss may not be recoverable (Awal and Ikeda, 2002).

Therefore, it might be possible that all genotypes tested are drought resistant. The assumption underlying the experiment is that, once drought tolerant genotypes were identified, the drought tolerant genotypes should possess some root characters and/or morpho-physiological traits that are related to drought resistance. Based on the results HI did not provide useful information, but other physiological traits may help.

Relative Water Content (RWC): The drought stress significantly reduced RWC and significant differences among peanut genotypes for RWC at 70 DAS were observed under water stress conditions (1/3 AW) only (Table 3). ICGV 98324 performed best for this character followed by ICGV 98353 and ICGV 98303, respectively and the results were in accordance with those for its DTI. Significant differences in RWC between water regimes were also found in all peanut genotypes, in which peanut genotypes grown under FC had significantly higher RWC

Table 3: Relative Water Content (RWC), Specific Leaf Area (SLA) and Drought Tolerance Index (DTI) under Field Capacity (FC) and available water (1/3 AW) conditions at 70 days after sowing

	RWC (%)			SLA ($cm^2 g^{-1}$)		
Genotypes	FC	1/3 AW	DTI	FC	1/3 AW	DTI
ICGV 98303	97.8	79.2ab*	0.81ab	184.0a	153.0a*	0.83
ICGV 98324	96.8	87.0a*	0.90a	156.8ab	153.6a	0.98
ICGV 98353	96.6	83.1a*	0.86a	143.5b	133.2b	0.94
KK 4	97.1	72.6b*	0.75b	176.4a	161.6a	0.93
Mean	97.1a	80.5b	0.83	165.2a	150.4b	0.92

For comparison among peanut genotypes, Mean values in the same column with the same latter(s) were not significantly different by LSD at $p \leq 0.05$. For water regime comparison, Means in the same row with the same capital ietter(s) were not significantly different by LSD at $p \leq 0.05$. For comparison the same genotype with difference two water treatments, Mean in the same row with the symbol (*) were significantly different by LSD at $p \leq 0.05$. DTI for genotype were calculated by the ratio of stressed (1/3 AW)/non stress (FC) conditions

than those grown under drought (Table 3). The visual wilting observed in genotypes exposed to water stress was also found at 70 DAS. However, peanut genotypes were not significantly different in RWC under drought at 50 and 60 DAS and the differences between water regimes at these evaluation dates were also not significant (data not showed). The results indicated that RWC was sensitive in identifying drought stress even in the same peanut genotypes with different water regimes in case of appropriate stress level. In case of mild drought stress, the use of RWC would not be appropriate because there was no significant difference between stressed and non-stressed treatments earlier than 7 DAS. This is possibly due to slow response of peanut to declining water level, whereas the response of soil to water depletion was more acute than peanut plants.

Relative water content in peanut is usually in a range of 30-100%, non-stressed plants have relative water content in a range of 85-100% (Reddy *et al*., 2003). According to Reddy *et al*. (2003), biochemical components in leaves of stressed plants were changed although the plants could maintain relative water content as high as those for non-stressed plants and relative water content in a range lower than 85% is considered severely stressed.

In general, the RWC was decreased markedly in response to declining soil water availability. The reduction was more pronounced in sensitive varieties. In this study relative water contents of peanut experienced drought treatment and well-watered treatment were not statistically different at 50 and 60 DAS and the significant difference occurred at 70 DAS (Fig. 1). Although soil water content showed significant difference between stressed and non-stressed treatments, plants showed similar relative water content at earlier than 70 DAS. The contrasting results were reported by Arunyanark *et al*. (2008) and Pimratch *et al*. (2008), who found significant difference in RWC between drought treatment and control treatment as early as 33-35 days after withholding water. The discrepancy of the results might be due to the difference in experimental conditions between greenhouse and field. Diurnal variation, leaf position and leaf age could affect RWC (Reddy *et al*., 2003). However, peanut genotypes were significantly different for RWC under drought conditions. ICGV 98324 had the highest RWC and should be a promising parental line for high RWC for drought resistance breeding.

Specific Leaf Area (SLA): Low SLA is preferable as it indicates higher drought resistance. Drought stress significantly reduced SLA (Table 3). Peanut genotypes were also significantly different in SLA at all water regimes and ICGV 98353 showed the most consistently lower SLA than other genotypes. However, ICGV 98303 was the only one genotype that showed significant reduction in SLA. Low SLA indicated thicker leaves and could be used as an economically surrogate trait for drought resistance. In the present study, peanut genotypes were not significantly different in DTI for SLA, indicating similar responses of peanut genotypes for SLA. However, Nageswara Rao *et al*. (2001) suggested that, if SLA is to be used as a screening tool, then sampling should be performed on clear (full sunlight) days. Under high-radiation condition, variation in SLA should be largely driven by photosynthetic capacity. Thus, genotypic differences in SLA as a consequence of photosynthetic capacity may be better expressed on days with high radiation. It could be hypothesized that peanut genotypes with low SLA have more photosynthetic machinery per unit leaf area and hence potential for greater assimilation under drought stress because thicker leaves usually have a greater photosynthetic capacity compared with thinner leaves.

Although, SLA was reduced by drought stress, SLA in certain peanut genotypes under drought stress was dependent on that under well-watered conditions. For example, ICGV 98353 showed consistently low SLA under both drought and well-watered conditions. The variation and consistency of SLA make it useful for use as a selection criterion in drought resistance breeding program.

SPAD Chlorophyll Meter Reading (SCMR): Difference in SCMR between water treatments was not significant and significant differences among peanut genotypes were found under field capacity only (Table 2). ICGV 98324 had the highest SCMR under field capacity. DTI for SCMR was also not significantly different among peanut genotypes, indicating similar responses of peanut genotypes for SCMR. However, the present study has also shown that, KK 4 trend to show high SCMR under drought stress conditions. Leaf photosynthesis is generally correlated with chlorophyll content per unit leaf area and hence the SPAD chlorophyll meter can provide a useful tool to screen for genotypic variation in potential photosynthetic capacity under drought conditions (Nageswara Rao *et al*., 2001; Songsri *et al*., 2008d).

Although, it is not significantly different between water regimes, drought seemed to increase SCMR. Similar to these results, Jongrungklang *et al.* (2008) found that drought significantly increased SCMR. The identification and use of surrogate traits for SCMR are simple and useful as a selection criterion for drought tolerance in peanut because of high heritability (Songsri *et al*., 2008b). Nageswara Rao *et al.* (2001) found that there were significant interrelationships among SLA, specific leaf nitrogen (SLN) and SCMR and they suggested that SCMR could be used as a reliable and rapid measure to identify genotypes with low SLA or high SLN (and hence high transpirastion efficiency) in breeding and peanut selection programmes. Nigam and Aruna (2008) suggested that SCMR and SLA can be recorded at any time after 60 days of the crop growth, preferably under moisture deficit conditions. However, as suggested by Serraj *et al.* (2004), these measurements should be recorded after imposition of moisture deficit and particularly at mid-way through stress.

Root Length (RL), Root Surface (SR), Root Volume (RV) and Root Dry Weight (RDW): Root characters other than RDW were not significantly different among peanut genotypes for both water regimes and difference between water regimes was also not significant (data not shown). The lack of variation in root characters might be due to the difficulty in recovering roots from soil and the limitation of root growth due to the confinement of roots in the containers. However, peanut genotypes were significantly different in RDW at 1/3 AW at 70 and harvest ($p \leq 0.10$), whereas at field capacity the differences among peanut genotypes were not significant (Table 4). The differences in DTI for RDW among peanut genotypes were significant at 70 DAS only. Drought stress also reduced RDW at 70 DAS but not at harvest. Increased RDW in response to drought stress was observed in peanut genotypes ICGV 98303 and KK 4. The increase in RDW in ICGV 98303 was found as early as 70 DAS, whereas the increase in RDW in KK 4 was found at harvest only. ICGV 98303 and KK 4 also showed the highest DTI for RDW. The observations showed that the varieties with low RWC tended to have a higher RDW indicating that drought stress would induce

Table 4: Root Dry Weight (RDW) and Drought Tolerance Index (DTI) under Field Capacity (FC) and available water (1/3 AW) conditions at 70 day after sowing and harvest

	RDW ($g\ plant^{-1}$) at 70 DAS			RDW ($g\ plant^{-1}$) at harvest		
Genotypes	FC	1/3 AW	DTI	FC	1/3 AW	DTI
ICGV 98303	1.26	1.40ab	1.11a	1.82	1.92a	1.20
ICGV 98324	1.90	1.40ab	0.74b	1.70	1.69b	0.99
ICGV 98353	1.55	1.28b	0.84b	1.84	1.77ab	0.94
KK 4	1.65	1.55a	0.95ab	1.70	1.75ab	1.10
Mean	1.59a	1.41b	0.91	1.76	1.78	1.05

For comparison among peanut genotypes, Mean values in the same column with the same latter(s) were not significantly different by LSD at $p \leq 0.10$. For water regime comparison, Means in the same row with the same capital letter(s) were not significantly different by LSD at $p \leq 0.10$. DTI for genotype were calculated by the ratio of stressed (1/3 AW)/non stress (FC) conditions

increased root production such as KK 4. Del Rosario *et al.* (1988) also reported that the varieties with low leaf water potential tend to have a higher RDW indicating that severe stress would induce increased root production. Songsri *et al.* (2008c) found that RLD in the deeper subsoil level was increased in response to drought and RLD under drought conditions was not related to biomass production. However, they found that the ability to maintain the percentage of RLD (DTI for RLD (%)) was related to pod yield, DTI for pod yield and DTI for HI. The ability of peanut to maintain a viable root system during water stress may contribute to the crop's drought resistance (Reddy *et al.*, 2003).

CONCLUSION

Drought stress reduced RWC, SLA, RDW and biomass production. Peanut genotypes were significantly different for SLA under water stress and well-watered conditions, but they were significantly different for RDW and RWC under water stress conditions only, indicating that drought stress increased variation for these traits. Peanut genotypes showed different responses for traits associated with drought resistance and the genotypes with good performance for traits associated with drought resistance could be identified. ICGV 98353 was a good genotype for SLA, whereas ICGV 98324 was a good genotype for RWC. KK 4 had high SCMR under drought stress conditions, whereas, ICGV 98303 had the highest DTI for RDW. Differential responses of peanut genotypes for these traits indicated that several drought resistance mechanisms might exist. Combining these characters in peanut breeding programs should increase drought resistance in peanut.

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Abstract: Improvement of terminal drought resistance in peanut can increase their productivity in drought-prone environments and reduce aflatoxin contamination. To improve selection efficiency for superior drought tolerant genotypes, a study of inheritance of traits is worthy, and provides useful information for plant breeders. The objectives of this study were to estimate the heritabilities of terminal drought resistant traits and the genotypic and phenotypic correlations between drought resistant traits and agronomic traits in peanut. The 140 peanut lines in the F4:6 and F4:7 generations were generated from four crosses, and tested under well-watered and terminal drought conditions. Field experiments were conducted under the dry seasons 2006/07 and 2007/08. Data were recorded for agronomic traits [biomass (BIO), pod yield (PY), number of mature pods per plant, seeds per pod, and seed size] and physiological traits [harvest index (HI), SPAD chlorophyll meter reading (SCMR), and specific leaf area (SLA)]. The heritabilities for physiological traits were higher than for agronomic traits, and varied among crosses. The heritability for HI, SCMR, and SLA ranged from 0.58 to 0.85, 0.66 to 0.91, and 0.61 to 0.90, respectively. Positive correlations between HI and SCMR with agronomic traits were found. SLA were also negatively correlated with agronomic traits. These results implied that HI, SLA, and SCMR are potentially useful as indirect selection traits for terminal drought resistance because of their high heritabilities, and significant correlations with pod yield. Plant breeding approaches using these traits might be effective and valuable for improving drought tolerance in peanut.

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

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Key word:

Arachis hypogaea L.

Heritability

Genotypic correlation

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1. 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 x 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).

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 a 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.

2. Materials and methods

2.1. 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.

2.2. 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}.

2.3. 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.

2.4. Data collection

2.4.1. 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 (Fig. 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.

2.4.2. 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.

2.4.3. 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 a.m. 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}).

2.4.4. 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:

$$\text{HI} = \text{pod weight} / \text{total biomass} \qquad (1)$$

2.5. Statistical analysis

Analysis of variance was performed for each trait in each year following a split plot design (Gomez and Gomez, 1984). Homogeneity of variance was tested for all characters and combined analysis of variance of two years data was performed where appropriate.

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):

$$h^2_b = \delta^2_G / \delta^2_P \quad (2)$$

$$\delta^2_P = \delta^2_G + \delta^2_{GE} /e + \delta^2_E /re \quad (3)$$

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 tolerant traits and agronomic traits, and correlations among physiological traits were calculated following the methods of Falconer and Mackay (1996) as follows (Table 1):

$$\text{Phenotypic correlation, } r_P = (M_3{*}\ M_3) /[(M_3{*}\ M_3)]^{1/2} \quad (4)$$

$$\text{Genotypic correlation, } r_G = (M_3{*}\ M_3 - M_2{*}\ M_2) / [(M_3{*} - M{*}_2) (M_3 - M_2)]^{1/2} \quad (5)$$

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.

3. Results and Discussions

3.1. 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) (Fig. 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.

3.2. 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.

3.3. 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 0.61 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 ($p = 0.34-0.44$, $P \leq 0.01$) (Table 3), indicating that these traits could be selected under either well-watered or terminal drought conditions.

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.

3.4. Phenotypic and genotypic correlations between drought resistant 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, $P \leq 0.01$). Positive correlations between SCMR and pod yield, biomass, and seed size were also significant ($r_G = 0.21$ to 0.35, $P \leq 0.01$). 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 ($r_G = -0.07$ to -0.34, $P \leq 0.05$ and $P \leq 0.01$, respectively). Negative correlations between SLA and pod yield and biomass under stressed conditions were found ($r_G = -0.12$ to -0.34, $P \leq 0.01$), 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 ($r_G = -0.07$ to 0.09, $P \leq 0.05$ and $P \leq 0.01$, 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.

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.

3.5. Genotypic correlations among drought resistant 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 tolerant 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 ($r_G = 0.15$, $P \leq 0.01$) and stressed ($r_G = 0.18$, $P \leq 0.01$) conditions. The SLA was found to be inversely associated with SCMR and HI. Under terminal drought, SLA was negatively correlated with HI ($r_G = -0.33$, $P \leq 0.01$) and SCMR ($r_G = -0.30$, $P \leq 0.01$). Under non-stressed conditions, negative correlation between SLA and SCMR was also observed ($r_G = -0.41$, $P \leq 0.01$). 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.

4. 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 programs.

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Table 1

Analysis of variance of cross and cross product.

Source of variation	Degrees of freedom	Mean square of character		MCP[†]	EMS[‡]	EMCP[§]
		X	Y			
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

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	1088**	344.3	21657*	524796**		
Rep. within Y	6	42	59.4	3381	3157		
Genotypes (G)	139	47**	52.6**	787**	1232**		
Y x G	139	7	10.2	199	410*		
Pooled error	834	7	8.9	209	288		

* and ** significant at P < 0.05 and significant at P < 0.01, respectively.

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	0.86± 0.14	0.83± 0.16
ICGV 98348 x KK 60-3	0.73± 0.20	0.52± 0.29	0.77± 0.18	0.87± 0.14	0.74± 0.20
ICGV 98353 x Tainan 9	0.60± 0.26	0.49± 0.30	0.65± 0.25	0.90± 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	0.75± 0.19
Drought conditions					
ICGV 98348 x Tainan 9	0.57± 0.27	0.53± 0.29	0.58± 0.27	0.75± 0.20	0.61± 0.25
ICGV 98348 x KK 60-3	0.75± 0.19	0.66± 0.23	0.85± 0.14	0.69± 0.22	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	0.74± 0.20
ICGV 98353 x KK 60-3	0.45± 0.31	0.36± 0.34	0.63± 0.25	0.73± 0.21	0.74± 0.20
Correlation (r)†	0.43**	0.40**	0.44**	0.34**	0.34**

* and ** significant at $P < 0.05$ and significant at $P < 0.01$, respectively.

† Correlations between well-watered conditions and drought conditions.

‡ Standard error.

1

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	0.35**	0.21**	0.02	-0.27**	0.30**
SLA	-0.04	0.00	0.09*	-0.02	-0.17**
Drought conditions					
HI	0.71**	-0.08*	0.78**	0.48**	0.05
SCMR	0.31**	0.30**	-0.08*	-0.09*	0.28**
SLA	-0.34**	-0.12**	-0.07*	-0.01	-0.21**

* and ** significant at $P < 0.05$ and significant at $P < 0.01$, respectively.

2

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**	0.18**	-0.02
SCMR		-0.30**		-0.41**

* and ** significant at P < 0.05 and significant at P < 0.01, respectively.

Figure captions

Fig. 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)

Fig. 2 Soil moisture volume fraction (a and c) at planting, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 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 difference water regimes [field capacity (FC) and 1/3 available water (1/3 AW)] averaged from 0-60 cm depth in 2006-2007 (a and b) and 2007/2008 (c and d)

Figure

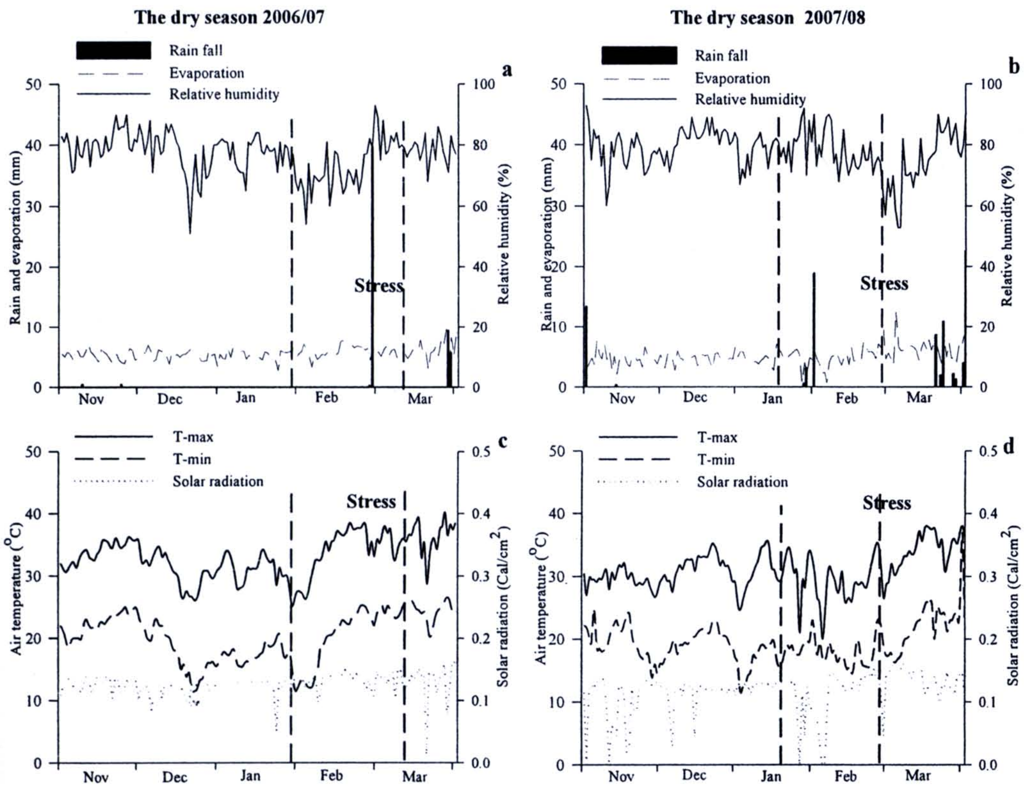

Fig. 1. Relative humidity (%) (a and b), pan evaporation (mm) (a and b), rainfall (mm) (a and b), maximum and minimum air temperature (°C) (c and d), and solar radiation (Cal/cm^2) (c and d) during the crop growth period in 2006/07 (a and c) and in 2007/08 (c and d)

Figure

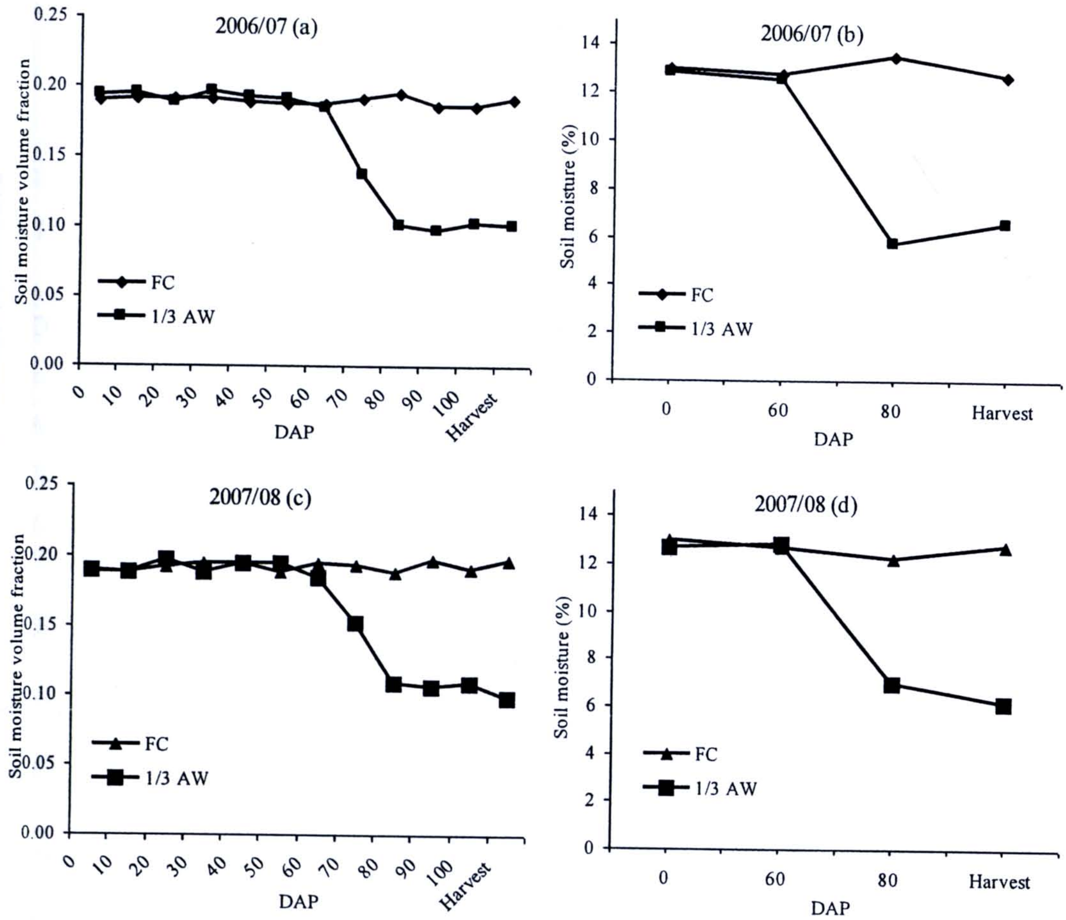

Fig. 2. Soil moisture volume fraction (a and c) at planting, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 days after planting (DAP), and at final harvest and gravimetric soil moisture content (b and d) at planting, 60, 80 DAP and at final harvest under difference water regimes [field capacity (FC) and 1/3 available water (1/3 AW)] averaged from 0-60 cm depth in 2006-2007 (a and b) and 2007/2008 (c and d)

Heritability of Early Season Drought Resistance Traits and Genotypic Correlation of Early Season Drought Resistance and Agronomic Traits in Peanut

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Abstract. Inheritance of drought tolerance traits should be known for formulating suitable breeding strategies to improve drought tolerance. Two field experiments were conducted both under early season drought (ESD) and irrigated conditions using 90 lines in the $F_{4:7}$ and $F_{4:8}$ generation from four peanut crosses. Data were recorded for specific leaf area (SLA), SPAD chlorophyll meter reading (SCMR), biomass, pod yield, harvest index (HI). Imposition of ESD followed by recovery resulted in an average increase in yield. The heritability estimate (h^2) for biomass and pod yield were low for all tested crosses while the h^2 for HI, SLA and SCMR were high for all four crosses indicating that breeding for drought resistance might be more advantageous than selecting for yield because of high h^2 and low G x E interaction. The HI had high genetic correlation (r_G) with pod yield and other agronomic traits under both irrigated and ESD conditions. In addition, SCMR after recovery had moderate r_G with number of mature pod, indicated that physiological response to recovery was most importance to yield than physiological response during drought. Hence, integrate selection based on these physiological traits might help to improve breeding progress.

Additional Keywords: *Arachis hypogaea* L., Inheritance, SCMR, Drought

Introduction

Drought is one of the important factors limiting the yield and quality of peanut worldwide. Yield of peanut is substantially reduced by drought stress at flowering and pod formation phases (Nageswara Rao *et al.* 1985; 1988; Nautiyal *et al.* 1999). However, drought stress during pre-flowering stage can increase yield in some peanut genotypes (Nageswara Rao *et al.* 1985; Nautiyal *et al.* 1999). A considerable increase in pod yield of 13-57% had been reported (Nageswara Rao *et al.* 1985; Nautiyal *et al.* 1999; Puangbut *et al.* 2010). This could be a novel strategy to increase peanut productivity through appropriate irrigation scheduling and opens an opportunity for breeding of peanut for increased yield under pre-flowering drought. Because peanut is usually grow under rainfed conditions (Wright and Nageswara Rao 1994). In Southeast Asia, most of peanut production area is under rainfed conditions (Jogloy *et al* 1992). Most of peanut is grown in the monsoon during late April and early May (Reddy *et al.* 2003). In this situation, drought is a recurring during pre-flowering phase. Therefore, selection of superior genotypes for high yield by exposing the peanut to early season drought or pre-flowering drought might be a promising strategy to improve pod yield. Previously, selection for superior genotypes has been done almost entirely based on final yield. Although this approach has been successful in increasing yields of various crops in the past, further progress is becoming more difficult due to high G x E interaction and low heritability (Araus *et al.* 2002; Richards *et al.* 2001) resulting in slow breeding progress (Wright *et al.* 1996). However, selection for the physiological traits might help to improve breeding progress if these traits have high heritability and low G x E interaction.

Modern breeding approaches utilizing physiological traits have been proposed to improve the efficiency of selection for superior drought tolerant genotypes (Cruickshank *et* *al.* 2004). More rapid progress may be achieved by using physiological traits such as harvest index (HI) and transpiration efficiency (TE) (Nigam *et al.* 2005). In addition, SLA and SCMR have been used as surrogate traits for TE in peanut (Wright *et al.* 1994; Sheshshayee *et al.* 2006; Nigam *et al.* 2005).

Transpiration efficiency (TE), defined as total biomass production per unit of water transpired, and is not an easy trait to measure. Therefore TE is not practical for use in large-scale breeding programs for improving drought tolerance. Transpiration efficiency has negative correlation with carbon isotope discrimination (Δ) (Hubick *et al.* 1986; Wright *et al.* 1994). Whilst measurement of Δ is rapid, it is an expensive technique and may not be feasible in large segregating breeding populations.

Specific leaf area is negatively related to SCMR and Δ and hence TE, over a wide range of cultivars and environments in peanut (Nageswara Rao *et al.* 2001; Wright *et al.* 1994; Nageswara Rao and Wright 1994). The subsequent investigation found that low SLA genotypes had greater photosynthetic capacity for unit leaf area (Nageswara Rao *et al.* 1995), and they suggested that the leaf thickness (low SLA) could be useful as a selection criterion for enhancing TE in peanut. However, under non stress conditions, there are a few published reports suggesting the predominant role of additive gene effects in SLA inheritance (Nigam *et al.* 2001). Recently, Songsri *et al.* (2008) reported that HI, SLA and SCMR had high heritability and positive genetic correlation with pod yield under long period drought conditions, and they suggested that SCMR is useful as a selection trait for drought resistance.

However, several adaptive mechanisms of plants in response to drought stress depend on timing, intensity, and duration of drought coupled with other location specific environmental factors (Nigam *et al.* 2003). The mechanisms of physiological responses to early season drought might be different from those for long period drought, which have been reported previously (Songsri *et al.* 2008). Puangbut *et al.* (2009*a*, 2010) revealed that early season drought followed by recovery result in an increase SCMR, Leaf area, N_2 fixation and yield. Hence, heritability estimates of physiological traits and pod yield evaluated under early drought might also be different, and the heritability estimates evaluated under early season drought and recovery are not understood. A better understanding of heritability estimate of physiological traits and pod yield under early season drought in peanut is important for crop improvement. The objective of this study was focused to estimate (i) heritability of drought resistant traits under early season drought and recovery (ii) genotypic and phenotypic correlations between drought resistant traits and agronomic traits under early season drought. Information on the inheritance of HI, SLA and SCMR will be useful for planning a suitable breeding strategy for improving early season drought tolerance (increase yield and low water use). It is expected that pod yield of peanut can be improved by reducing water supplied to the crop during the pre-flow phase.

Materials and methods

Genetic materials

Four peanut F_1 hybrids (ICGV 98300 x KK 60-3, ICGV 98300 x Tainan 9, ICGV 98303 x Tainan 9, and ICGV 98305 x Tainan 9) were generated from the hybridization of three drought resistant lines (ICGVs 98300, 98303 and 98305) with two high yielding cultivars KK 60-3 and Tainan 9 which were released cultivars from Thailand. ICGVs 98300 and 98303 are having high increasing in yield and ICGV 98305 having low increasing in yield under early season drought followed by recovery (Puangbut *et al.* 2009*a*). KK 60-3 and ICGV 98303 having high SCMR and low SLA (Puangbut *et al.* 2009*b*; Songsri *et al.* 2009). ICGVs 98300 and 98305 having moderate SLA and SCMR. Tainan 9 is having high SLA and low SCMR (Puangbut *et al.* 2009*b*; Songsri *et al.* 2009). The F_1 seeds were planted and their seeds harvested in bulk for each cross. In F_2 and F_3 generations, two pods were kept from each plant and remaining was bulked. Line separation was carried out in the F_4 generation. A total of 90 lines (20 and 25 lines for each cross) were randomly selected and multiplied in the F_5 and F_6 generation.

The 90 lines from four crosses were evaluated in the $F_{4:7}$ and $F_{4:8}$ generations (F_4–derived lines in the F_7 and F_8 generations, respectively) under two soil moisture levels (field capacity (FC) and 1/3 available soil water (1/3 AW)) for two years in dry season 2006/07 and 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) during December 2006 to April 2007, and repeated during December 2007 to April 2008. Soil type is Yasothon Series (loamy sand, Ocix Paleustults) with the soil moisture of FC is 11.2% and permanent wilting point is 4.8%. Two soil moisture levels FC (11.2%), and 1/3 AW (6.9%) 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 a length of 3 m. Spacing was 40 cm between rows and 20 cm between hills within the row.

Crop management

Land was thoroughly prepared for planting by ploughing three times. Lime (625 kg ha^{-1}), Urea (40.8 kg N ha^{-1}), phosphorus as triple superphosphate (24.7 kg P ha^{-1}) and potassium as potassium chloride (31.1 kg K ha^{-1}) were applied prior to planting. Seeds were treated with Captan (3a, 4, 7, 7a-tetrahydro-2-[(trichloromethyl)thio]-1H-isoindole-1, 3(2H)-dione) @ of 5 gm kg^{-1} seed before planting, and seeds of the large seeded genotypes were also treated with Ethel solution @ 2 mL L^{-1} water to break dormancy. Seeds were treated with water-diluted commercial peat-based inoculum of *Bradyrhizobium* (mixture of strains THA 201 and THA 205; Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand) on the rows of peanut plants. Weeds were controlled by an application of Alachlor (2-cholro-2', 6'-diethyl-*N*-(methoxymethyl) Acetanilide 48%, w v^{-1}, emulsifiable concentrate) @ 3 L ha^{-1} at planting and hand weeding later. Three to four seeds were sown per hill and the seedlings were thinned to one plant per hill at 7 day after emergence (DAE). Gypsum ($CaSO_4$) @ 312 kg ha^{-1} was applied at 40 DAE. 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^{-1}, water soluble concentrate] @ 2.5 L ha^{-1}, methomyl [*S*-methyl-*N*-((methylcarbamoyl) oxy) thioacetimidate 40 % soluble powder] @ 1.0 kg ha^{-1} and carboxin [5, 6-dihydro-2-methyl-1, 4-oxath-ine-3-carboxanilide 75 % wettable powder] @ 1.68 kg ha^{-1}.

A subsoil-drip-irrigation system (Super Typhoon®, Netafim Irrigation equipment & Drip systems, Israel), 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 at mid way between peanut rows and fitted with a pressure valve and water meter to supply a uniform measured amount of water across each plot. Soil moisture was initially maintained at field capacity (FC) to a depth of 20 cm (32.48 mm) to facilitate uniform emergence. After 8 day after planting, the early season drought treatment was imposed by withholding water until soil moisture reached a level of 1/3 available water (AW), after which soil moisture was maintained at 1/3 AW level until 40 DAE. 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 of Doorenbos and Pruitt (1992) and Singh and Russell (1981), respectively.

Total crop water use for each water treatment was calculated as the sum of transpiration and soil evaporation. Transpiration was calculated using the methods described by Doorenbos and Pruitt (1992):

$$ET_{crop} = ET_o \times K_c,$$

where ET_{crop} = crop water requirement (mm/day), ET_o = evapotranspiration of a reference plant under specified conditions calculated by pan evaporation method, Kc = the crop water requirement coefficient for peanut, which varies with genotype and growth stage (Doorenbos and Kassam 1986). Surface evaporation (E_s) was calculated as (Singh and Russell 1981):

$$E_s = \beta \times (E_o/t),$$

where E_s = soil evaporation (mm), β = light transmission coefficient measured depending on crop cover, E_o = evaporation from class A pan (mm/day), t = days from the last irrigation or rain.

Data collection

Weather parameters

The field trials were planted during the dry seasons of November 2006 to March 2007 and December 2007 to April 2008. There was maximum rainfall (39.7 mm) at 75 DAE in the dry season 2006/07, and 22.6 mm at 110 DAE in the dry season 2007/08 (Fig. 1). The seasonal mean maximum and minimum air temperature ranged between 33.1°C and 20.0°C during 2006/07 and 31.7°C and 19.7°C during 2007/08. Daily pan evaporation ranged from 1.62 to 9.84 mm (2006/07) and 1.78 to 12.68 mm in (2007/08). The seasonal mean solar radiation of 18.7 MJ m^{-2} d^{-1} (2006/07) and, 18.0 MJ m^{-2} d^{-1} (2007/08) were observed during the growing period.

Soil moisture status

Soil moistures were measured by gravimetric method during planting and harvesting to the depths of 0-5, 25-30 and 55-60 cm. The measurement at planting was used for calculating the correct amount of water to be applied to the crop, and the measurement at harvest was utilized for calculating the water use of the crop. The soil water status was also monitored at 7-day intervals using a neutron moisture meter (Type I.H. II SER. N° N0152, Ambe Diccot Instruments Co. Ltd., England). Sixteen-second neutron moisture meter readings were made once in a week from a depth of 0.3 m to 0.6 m at 0.3-m intervals.

SPAD chlorophyll meter reading (SCMR) and specific leaf area (SLA)

In each plot, SPAD Chlorophyll meter readings were recorded on five randomly selected plants. Specific leaf area measurements were made at 40 and 60 day after emergence (DAE) following the procedure described by Nageswara Rao *et al.* (2001). The second fully-expanded leaves were detached from the chosen plants between 8.30 and 9.30 a.m. and brought to the laboratory in closed polythene bags for recording observations. The SPAD Chlorophyll meter (Minolta SPAD-502 meter, Tokyo, Japan) reading (SCMR) was recorded twice on each leaflet of the tetrafoliate leaf along the mid-rib. While recording the SCMR, care was taken to ensure that the SPAD meter sensor fully covered the leaf lamina and that the 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). The measured leaves were then dried in an oven at 80°C for at 48 hours to determine the leaf dry weight. The SLA was derived as leaf area per unit leaf dry weight (cm^2 g^{-1}). The SLA was calculated using the following formula:

$$SLA = \text{Leaf area } (cm^2) / \text{Leaf dry weight } (g)$$

Agronomic traits

In each plot, three rows of plants present in 2.8 m length (3.4 m^2) were harvested during maturity (R8) (Boote 1982). The pods were removed before taking shoot fresh weight in the field. Two kg of random sample of shoots was oven-dried at 80°C for 48 hours and dry weight was measured. Shoot dry matter content was then calculated and used in determining shoot dry weight for a plot. Pod yield was measured after air drying to 8 % moisture content.

The number of matured pods per plant (matured pods was separated from immature pods, which were identified by dark internal pericarp color), number of seed per pod and 100 seed weight were also recorded during harvest.

Harvest index was computed by the following formula:

HI = Total pod dry weight at the last harvest / Total biomass at the last harvest.

Drought tolerance index (DTI) was calculated for biomass and pod yield, as suggested by Nautiyal *et al.* (2002), using the relationship as follows:

DTI = stress treatment / non-stress treatment

Statistical analysis

Individual analysis of variance was performed for each year following a split plot design (Gomez and Gomez 1984). Homogeneity of variances was tested for all characters and combined analysis of variance of two-year data was performed. Statistical analysis was carried out using MSTAT-C package (Bricker 1989).

Data for each cross and each water regime were analyzed separately according to randomize complete block design (RCBD). The estimations of variances and covariance components were performed for each cross that composes the estimates of heritability for all traits. The broad-sense heritability was calculated in four crosses by partitioning the variance components of genotype mean squares to pooled environmental variance (σ^2_E) genotype by environment interaction variance (σ^2_{GE}) and genotypic variance (σ^2_G), The formula to calculate broad-sense heritability is as follows (Holland *et al.* 2003):

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

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

where: h^2_b = broad sense heritability, σ^2_G = genotypic variation, σ^2_P = phenotypic variation, σ^2_{GE} = genotype x environment interaction, σ^2_E = experimental error, r = no. of replications, and e = no. of environments. The standard error (SE) of heritability (Singh *et al.* 1993) for drought resistance traits were calculated to give a precision measure of the estimate.

The heritability estimates was measured in late generations (F_7 and F_8) where 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 resistant traits and agronomic traits were calculated as suggested by Falconer and Mackay (1996) (Table 1).

$$\text{Phenotypic correlation } (r_P) = (M^*_3 M_3) / [(M^*_3)(M_3)]^{1/2}$$

$$\text{Genotypic correlation } (r_G) = (M^*_3 M_3 - M^*_2 M_2) / [(M^*_3 - M^*_2)(M_3 - M_2)]^{1/2}$$

where M is mean square of character *X* and M* is mean square of character *Y*. Simple correlation was used to determine the relationship between biomass, pod yield, and drought resistant traits under irrigated and ESD conditions to understand the consistent performance of peanut genotypes across environments.

Results

Monitoring of soil moisture

Soil moisture was measured using a neutron moisture meter at 7-day intervals until harvest (Fig. 2). The results showed a clear distinction between different soil moisture levels noted at 30 and 60 cm of soil depth. Under the early season drought (ESD) treatment, soil moisture was reduced during 0-40 days after emergence (DAE). However after re-watering, soil moisture increased to a range similar to that for irrigated treatment until at harvest in both seasons.

Combined analysis of variance

Combined analysis of variance showed significant differences among 90 progenies ($P \leq 0.01$) for biomass production, pod yield, and the drought resistant traits HI, SCMR, and SLA (Table 2). This indicated that genetic variation exists for these characters, and, thus, heritability could be estimated. The interaction effects of Y x G were significant ($P \leq 0.01$) for pod yield, biomass under irrigated and ESD conditions but not significant for HI, SCMR and SLA in both irrigated and ESD conditions. Wright *et al.* (1996) reported that pod yield is a complex trait in which multiple genes are involved, and high G x E interaction is expected. Based upon low coefficient of variation (CV) and high F-ratio from analysis of variance the best assessment time for SLA and SCMR was fixed as at 40 DAE during water stress imposed and 60 DAE after re-watering.

The means and range of all traits for all four peanut crosses are presented in Table 3. The means of pod yield and biomass were higher for all four peanut crosses under ESD conditions, pod yield ranging from 1.8 to 2.9 t ha^{-1}, and 5.7-8.1 t ha^{-1} for biomass. The means of DTI (PY) and DTI (BIO) ranged from 1.2-1.6, while HI was rather high for all four peanut crosses under ESD conditions. At 40 DAE, ESD increased SCMR while SLA was decreased for all four peanut crosses. After recovery (60 DAE), SCMR was slightly increased, while SLA was still decreased under ESD conditions.

Heritability of drought resistance traits

Heritability estimates of four peanut crosses were calculated for biomass, pod yield, HI, DTI (BIO), and DTI (PY) at harvest and for SCMR and SLA at 40 and 60 DAE (Table 4). Heritability estimates for HI was high for all four peanut crosses under both irrigated and ESD conditions, ranging from 0.74 to 0.89. Drought tolerance indices for pod yield and biomass showed higher heritability estimates than those for pod yield and biomass themselves under the irrigated and ESD conditions. Heritability estimates for biomass and pod yield varied from 0.13 to 0.48 and DTI (BIO) and DTI (PY) varied from 0.42 to 0.59.

Heritability estimates for SLA and SCMR showed low standard error of estimation for all four peanut crosses under both irrigated and ESD conditions (Table 4). Heritability for SLA and SCMR at 40 DAE ranged from 0.83 to 0.99 and 0.84 to 0.98 for the irrigated and ESD treatments, respectively. After re-watering, at 60 DAE, heritability estimates for SLA and SCMR were still high, ranging from 0.87 to 0.99 and 0.68 to 0.99 for irrigated and ESD treatments, respectively.

Genotypic correlation among drought resistance traits

Phenotypic and genotypic correlations provided similar information in this study and hence only genotypic correlations are reported. Moderate and negative genotypic correlations were found between SLA and SCMR at 40 DAE under both ESD and irrigated conditions (-0.59, $P \leq 0.01$ and -0.52, $P \leq 0.01$, respectively), at 60 DAE, SLA had high negative correlation with SCMR under both ESD and irrigated conditions (-0.60, $P \leq 0.01$ and -0.63, $P \leq 0.01$, respectively) (Table 5). The DTI (BIO) was related inversely to SLA under ESD conditions at both 40 and 60 DAE (-0.54, $P \leq 0.01$ and -0.79, $P \leq 0.01$, respectively). High and positive genotypic correlations were found between SCMR under early drought (40 DAE) and recovery (60 DAE) (0.64, $P \leq 0.01$). The correlation between SLA under early drought (40 DAE) and recovery (60 DAE) was high (0.67, $P \leq 0.01$) (Table 5).

Genotypic correlation between drought resistance traits and yield and yield components

Genetic correlations between drought resistance traits and yield and yield components will provide information about these relationships and pave the way for easier selection. High genotypic correlations were found for HI and pod yield under both ESD (0.54, $P \leq 0.01$) and irrigated (0.76, $P \leq 0.01$) conditions (Table 6). The genotypic correlations between HI and number of mature pods per plant were moderate and positive under both ESD and irrigated treatments (0.48, $P \leq 0.01$ and 0.32, $P \leq 0.01$, respectively). Under ESD conditions, SCMR at both 40 and 60 DAE showed quite low positive correlations with biomass and pod yield while under irrigated conditions, SCMR at both 40 and 60 DAE showed strong positive correlations with biomass. SCMR at both 40 and 60 DAE had a strong and positive correlation with number of mature pods under the irrigated conditions (0.55, $P \leq 0.01$ and 0.61, $P \leq 0.01$, respectively), whereas the correlations between SCMR and number of mature pods was moderate and positive under the ESD conditions (0.32, $P \leq 0.01$and 0.46, $P \leq 0.01$, respectively).

A comparison of drought resistant traits (SLA and SCMR) under irrigated versus drought conditions should provide a better understanding of the most suitable conditions for selecting drought resistant genotypes. Significant correlations between traits (SLA and SCMR) under ESD and irrigated conditions were found in all four peanut crosses for SCMR (r = 0.59-0.71, $P \leq 0.01$) and SLA (r = 0.55-0.75, $P \leq 0.01$) (data not presented).

Discussion

The analysis of variance revealed that significant genotypic variation for all traits (BIO, PY, DTIs, HI, SCMR and SLA) which could potentially be exploited in peanut breeding programs (Table 2). The present study supports earlier findings that early season drought followed by recovery can result in higher yield of peanut compared with well-watered conditions (Puangbut *et al.* 2009a, 2010), while mid season drought or terminal decrease yields (Nageswara Rao et al., 1989). This could be possibility to select peanut genotypes with high yield under early season drought.

The results indicated that most characters had similar heritability estimates when compared between ESD and irrigated treatments (Table 4). This should make selection for drought tolerant easier, hence, selection for these characters can be carried out either under well-watered or drought conditions. The moderate heritability estimates for DTI based on pod yield and biomass suggested that this trait might be useful as a selection criterion for early season drought tolerance in peanut breeding programs. However, as heritability estimates for pod yield and biomass were rather low (Table 4), selection for these traits would not be effective because of low heritability and high G x E interaction. Previous reports indicated that selection for yield under stress conditions could not be effective (Araus *et al.* 2002). They thought that it might lead to poor repeatability of the results due to the large G x E interaction. Additional or improved selection criteria and procedures are needed.

Physiological traits have been used to identify traits linked with yield and many contribute to improvements in the efficiency of breeding (Cruickshank *et al.* 2004). The selection of the physiological traits such as SLA and SCMR might help to speed up breeding progress. Recently, Songsri *et al.* (2008) suggested that SLA and SCMR could be useful as selection criteria for drought resistant traits under long period drought. However, the heritability estimates for these traits under early season drought is not understood.

The high heritability estimates for SLA and SCMR under early season drought (ESD) (40 DAE) and recovery conditions (60 DAE) (Table 4) suggested that these traits could be used as selection criteria for identifying genotypes with rapid recovery from early season drought. Puangbut *et al* (2009*a*) indicated that physiological responses to recovery from early season drought were more importance in determining yield than that physiological response during drought. Several researchers reported that SCMR and SLA had low G x E interactions (Nigam *et al.* 2005, Krishnamurthy *et al.* 2007, Nigam and Aruna 2008; Songsri *et al.* 2008), indicating that these traits are stable across a wide range of environments. The information on heritability of HI, SCMR and SLA under early season drought following recovery is needed for predicting progress of selection.

Most of the drought resistant traits (HI, SCMR and SLA) in our study had high heritability and low G x E interaction (Table 4), indicating that breeding progress could be easily achieved for these characters. SCMR is easy to measure and is potentially useful as a selection trait for drought resistance (Nigam and Aruna 2008; Songsri *et al.* 2008). In subsequent attempts to breed for drought tolerances in peanut, SLA and SCMR have been identified as surrogate traits for improve TE (Nigam *et al.* 2005; Sheshshayee *et al.* 2006; Krishnamurthy *et al.* 2007). Our study revealed that a significant between DTI (BIO) and SLA (thus TE) highlights the importance of TE in crop productivity under water- limited conditions.

The simple correlation between SLA and SCMR were reported under the end of season drought conditions (Nigam and Aruna 2008) and under long period drought (Songsri *et al.* 2008). Our finding show that genotypic and phenotypic correlations between SLA and

SCMR were consistent under both irrigated and ESD conditions. In addition, great genotypic correlation of SCMR and SLA was found between evaluation at 40 DAE (drought period) and 60 DAE (recovery period) (Table 5), indicating the stability of the correlation throughout growth. Hence, the suitable time for evaluation of these characters might be assessed at only recovery period (60 DAE) due to physiological response to recovery was of most importance to yield than physiological response during drought (Puangbut *et al* 2009*a*, 2009*b*; Anyia and Herzog 2004). However, the differences of physiological response among peanut varieties were not significant after 60 DAE because the drought treatment was recovered, and the performance of the varieties after recovery was similar for SCMR and SLA (Puangbut *et al.* 2009, 2010). Therefore, the differences between the correlation of SCMR or SLA with yield at 60 DAE and after 60 DAE were not significant.

Although the correlation between SCMR after recovery with pod yield was positive under ESD conditions (r = 0.29) (Table 6), they were not high enough to make significant contribution to yield under early season drought. While, the correlations between SCMR and pod yield was highest under irrigated treatment. However, the relationship between SCMR under the ESD and irrigated conditions was correlated well (data not presented), it is advisable to first select peanut genotypes under irrigated conditions in large segregating populations because drought simulation is much more difficult, later, the selections can be refined under both drought and non-stress conditions in more advance generations (Songsri et al. 2008).

Among drought resistant traits studied [(DTI (BIO), DTI (PY), HI, SCMR and SLA], HI had the highest correlation with pod yield (Table 6). However, this correlation is autocorrelation, hence, HI is not only useful as criteria selection for improve yield. Thus, the use of integrated selection based on other physiological traits might help to improve yield.

SCMR after recovery had moderate correlation with number of mature pod, indicating the possibility of selection for this trait to improve yield. In addition, SCMR and HI have lower G x E interaction than that yield (Wright *et al.* 1996, Songsri *et al.* 2008). It would be possible to improve yield by selecting for high HI and SCMR. The SCMR is an indicator of the photo-synthetically activity light-transmittance characteristics of leaves and positively correlated with chlorophyll content (Akkasaeng *et al.* 2003) and chlorophyll density (Arunyanark *et al.* 2008). Also the findings of Nageswara Rao *et al.* (1995) reported that high SCMR genotypes having greater photosynthetic capacity further strengthened the suggestion of using high SCMR or low SLA as a selection criterion for enhancing TE in peanut.

In the subsequent efforts to breed for drought tolerance in peanut, high SCMR, as a surrogate for high TE, was incorporated into genotypes with high harvest index (partitioning to pod yield) (Nigam *et al.* 2005). It is expected SPAD chlorophyll meter could provide an easy opportunity to integrate a surrogate measure of TE with pod yield in the selection scheme of early season drought resistance breeding program in peanut. As drought resistance is a complex phenomenon, involving many mechanisms (Thomas *et al.* 1996), combinations of several traits as selection criteria for drought resistance might be more useful than the use of single traits.

4. Conclusion

HI, SCMR and SLA traits measured in these four peanut crosses had high heritability and low G x E interaction indicating the scope of drought tolerance studies in peanut. The selection of the physiological traits such as SLA and SCMR might help to speed up breeding progress. The findings indicated that SCMR is useful as a selection criterion for early season drought tolerance in peanut breeding programs. The present study revealed that

HI has a great correlation with yield and agronomic traits than that SCMR and SLA. However, SCMR should be more applicable in breeding program with large population because of simplicity in practice. Thus, the use of integrated selection based on these physiological traits might help to improve breeding progress.

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Table 1. Analysis of variance of cross and cross product

MCP, Mean square of cross product; EMS, Expected mean square; EMCP, Expected mean square of cross product; r, Number of replication

Source of variation	Degree of freedom	Mean square of Character		MCP	EMS	EMCP
		X	Y			
Year (Y)	Y-1					
Rep. within Y	Y(r-1)					
Genotypes (G)	G-1	M_3*	M_3	$M^*{}_3 M_3$	$\sigma^2_E + r\,\sigma^2_{FE} + re\,\sigma^2_F$	$\sigma_{E \cdot E} + r\,\sigma_{FE \cdot FE} + re\sigma_{F \cdot F}$
G x Y	(G-l)(Y-1)	M_2*	M_2	$M^*{}_2 M_2$	$\sigma^2_E + r\,\sigma^2_{FE}$	$\sigma_{E \cdot E} + r\sigma_{FE \cdot FE}$
Pooled error	Y(r-1)(G-1)	M_1*	M_1	$M^*{}_1 M_1$	σ^2_E	$\sigma_{E \cdot E}$

Table 2. Mean squares from the combined ANOVA for pod yield, biomass and drought tolerance index for biomass (DTI (BIO)) and pod yield (DTI (PY)) and harvest index (HI) at harvest, SPAD chlorophyll meter reading (SCMR) and specific leaf area (SLA) during water stress (40 DAE) and re-watering (60 DAE) under the irrigated and early season drought (ESD) of 90 lines in the dry season of 2006/07 and 2007/08

* Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$

DTI were calculated by the ratio of ESD (1/3 available water) / Irrigated (field capacity) conditions

Source of		Pod yield			Biomass			HI	
Variation	df	Irrigated	ESD	DTI(PY)	Irrigated	ESD	DTI (BIO)	Irrigated	ESD
Year	1	290.07	419.07	1.60	1606.62	1763.16	0.71	0.0123	0.06
Rep. within Y	6	0.48	1.61	0.44	2.16	6.05	0.23	0	0.01
Genotypes	89	1.58**	2.62**	0.64**	6.89**	10.17**	0.22**	0.01**	0.01**
Y x G	89	1.12**	0.85**	0.70**	3.58**	3.30**	0.20**	0.00	0.00
Pooled error	534	0.10	0.13	0.15	0.56	0.58	0.06	0.00	0.00

Source of		SLA 40 DAE		SCMR 40 DAE		SLA 60 DAE		SCMR 60 DAE	
Variation	df	Irrigated	ESD	Irrigated	ESD	Irrigated	ESD	Irrigated	ESD
Year	1	6964.53	6180.54	75.4	131.41	9.11	132.10	13.20	50.82
Rep. within Y	6	540.63	87.28	6.10	14.81	137.46	887.88	1.27	5.81
Genotypes	89	1189.39**	1718.53**	67.63**	29.98**	1334.02**	1025.44**	36.97**	48.70*
Y x G	89	177.36	91.49	1.63	1.41	37.42	134.17	1.51	1.68
Pooled error	534	150.36	71.96	2.37	1.73	80.51	143.43	1.81	2.4

Table 3. Means and range for pod yield, biomass, harvest index (HI), SLA and SCMR in 4 crosses of peanut under irrigated (IRR) and early season drought (ESD) conditions and DTI for pod yield (DTI (PY)) and biomass (DTI (BIO)).

	Pod yield (t ha^{-1})			Biomass (t ha^{-1})			HI		SCMR 40 DAE		SLA 40 DAE		SCMR 60 DAE		SLA 60 DAE	
Crosses	IRR	ESD	DTI	IRR	ESD	DTI	IRR	ESD	IRR	ESD	IRR	ESD	IRR	ESD	IRR	ES
ICGV 98300 X KK60-3																
Mean	2.2	2.9	1.6	6.4	8.1	1.3	0.32	0.33	43	46	181	166	41	45	179	16
Range	1.7-3.6	1.9-4.5	1.1-2.5	4.5-7.9	6.5-9.4	0.9-1.8	0.28-.035	0.28-.038	40-45	44-48	164-209	144-196	39-43	42-49	154-212	13
ICGV 98300 X Tainan9																
Mean	1.3	1.8	1.4	4.9	5.7	1.2	0.29	0.32	39	45	194	169	36	40	183	15
Range	1.1-1.6	1.3-2.1	1.1-1.8	4.2-5.6	4.5-6.9	1.0-1.5	0.25-0.33	0.29-0.35	33-42	42-48	170-209	153-189	34-39	36-43	168-199	14
ICGV 98303 X Tainan9																
Mean	1.5	1.8	1.3	5.2	5.8	1.2	0.30	0.33	35	39	194	165	38	44	188	16
Range	1.1-2.1	1.1-2.5	1.0-1.6	3.6-6.4	4.0-7.0	0.9-1.5	0.24-0.34	0.26-0.36	32-39	35-42	178-215	141-204	34-43	41-47	170-202	14
ICGV 98305 X Tainan9																
Mean	1.6	2.1	1.3	5.4	6.3	1.2	0.31	0.33	39	44	180	160	38	43	175	15
Range	1.2-2.2	1.4-2.6	1.0-1.9	4.5-6.5	5.4-7.8	1.0-1.7	0.26-0.34	0.26-0.36	35-44	41-46	154-205	145-178	35-42	39-46	160-186	14

Table 4. Estimates of heritability with standard error for biomass, drought tolerance index for biomass (DTI (BIO)), pod yield, drought tolerance index for pod yield (DTI (PY)), harvest index (HI), specific leaf area (SLA) and SPAD chlorophyll meter reading (SCMR) of 4 crosses of peanut under the early season drought (ESD) and irrigated conditions in the dry seasons of 2006/07 and 2007/08

DTI were calculated by the ratio of ESD (1/3 available water) / non-stressed (field capacity) conditions

	Heritability								
Cross	BIO	DTI (BIO)	PY	DTI (PY)	HI	SLA 40 DAE	SCMR 40 DAE	SLA 60 DAE	SCMR 60 DAE
ESD									
ICGV 98300 X KK60-3	0.30 ± 0.28	0.52 ± 0.31	0.48 ± 0.28	0.42 ± 0.30	0.82 ± 0.11	0.93 ± 0.08	0.90 ± 0.06	0.96 ± 0.06	0.92 ± 0.07
ICGV 98300 X Tainan9	0.23 ± 0.28	0.58 ± 0.29	0.20 ± 0.25	0.48 ± 0.23	0.80 ± 0.09	0.84 ± 0.10	0.97 ± 0.07	0.68 ± 0.15	0.94 ± 0.04
ICGV 98303 X Tainan9	0.45 ± 0.33	0.51 ± 0.30	0.45 ± 0.33	0.59 ± 0.27	0.89 ± 0.08	0.98 ± 0.03	0.98 ± 0.05	0.92 ± 0.07	0.95 ± 0.05
ICGV 98305 X Tainan9	0.18 ± 0.27	0.52 ± 0.30	0.20 ± 0.24	0.58 ± 0.28	0.74 ± 0.13	0.93 ± 0.08	0.96 ± 0.08	0.83 ± 0.12	0.99 ± 0.03
Irrigated									
ICGV 98300 X KK60-3	0.19 ± 0.28	—	0.23 ± 0.20	—	0.85 ± 0.11	0.92 ± 0.06	0.91 ± 0.05	0.95 ± 0.07	0.87 ± 0.07
ICGV 98300 X Tainan9	0.15 ± 0.20	—	0.20 ± 0.29	—	0.89 ± 0.08	0.83 ± 0.12	0.96 ± 0.05	0.97 ± 0.05	0.89 ± 0.07
ICGV 98303 X Tainan9	0.22 ± 0.24	—	0.21 ± 0.29	—	0.85 ± 0.09	0.91 ± 0.05	0.99 ± 0.02	0.98 ± 0.04	0.98 ± 0.03
ICGV 98305 X Tainan9	0.13 ± 0.20	—	0.19 ± 0.25	—	0.74 ± 0.12	0.88 ± 0.11	0.98 ± 0.04	0.98 ± 0.05	0.99 ± 0.02

Table 5. Genotypic (r_G) correlation estimates among drought resistance traits for all 4 peanut crosses of 90 lines in the dry seasons of 2006/07 and 2007/08 (degree of freedom = 356)

* Significant at $P \leq 0.05$, ** Significant at $P \leq 0.01$

ESD, early season drought; DAE, days after emergence

DTI were calculated by the ratio of ESD (1/3 available water) / Irrigated (field capacity) conditions

	DTI (PY)	40 DAE		60 DAE		HI
ESD		SCMR	SLA	SCMR	SLA	
DTI (BIO)	0.82**	0.17**	-0.54**	0.16**	-0.79**	-0.22**
DTI (PY)		0.13*	0.28**	0.29**	0.25**	0.41**
SCMR 40 DAE			-0.59**	—	—	0.25**
SLA 40 DAE				—	—	-0.12*
SCMR 60 DAE		0.64**		—	-0.60**	0.23**
SLA 60 DAE			0.67**			-0.10
Irrigated						
DTI (BIO)	—	—	—	—	—	—
DTI (PY)		—	—	—	—	—
SCMR 40 DAE			-0.52**	—	—	0.10
SLA 40 DAE				—	—	-0.27**
SCMR 60 DAE		0.72**			-0.63**	0.20**
SLA 60 DAE			0.48**			-0.22**

Table 6. Genotypic (r_G) correlation estimates between drought resistance traits and agronomic traits for all 4 peanut crosses of 90 lines in the dry seasons of 2006/07 and 2007/08 (degree of freedom = 356)

* Significant at $P \leq 0.05$ ** Significant at $P \leq 0.01$

ESD, early season drought; DAE, days after emergence

DTI were calculated by the ratio of ESD (1/3 available water) / Irrigated (field capacity) conditions

	Agronomic traits				
Drought resistance traits	Biomass (BIO)	Pod yield (PY)	Seed size	No. mature pods/plant	Seed/pod
ESD					
DTI (BIO)	0.58**	0.50**	0.22**	0.35**	0.12*
DTI (PY)	0.42**	0.53**	0.13*	0.42**	0.12*
SCMR 40 DAE	0.40**	0.32**	0.04	0.32**	-0.02
SLA 40 DAE	0.25**	0.20**	0.08	0.04	-0.35**
SCMR 60 DAE	0.22**	0.29**	-0.20**	0.46**	0.25**
SLA 60 DAE	0.16**	0.25**	0.21**	-0.10	-0.26**
HI	0.20**	0.54**	0.15**	0.48**	0.07
Irrigated					
SCMR 40 DAE	0.51**	0.65**	0.21**	0.55**	0.37**
SLA 40 DAE	-0.46**	-0.68**	-0.34**	-0.10	-0.49**
SCMR 60 DAE	0.43**	0.61**	0.16**	0.61**	0.34**
SLA 60 DAE	0.22**	0.22**	0.17**	-0.09	-0.29**
HI	0.29**	0.76**	0.50**	0.32**	0.53**

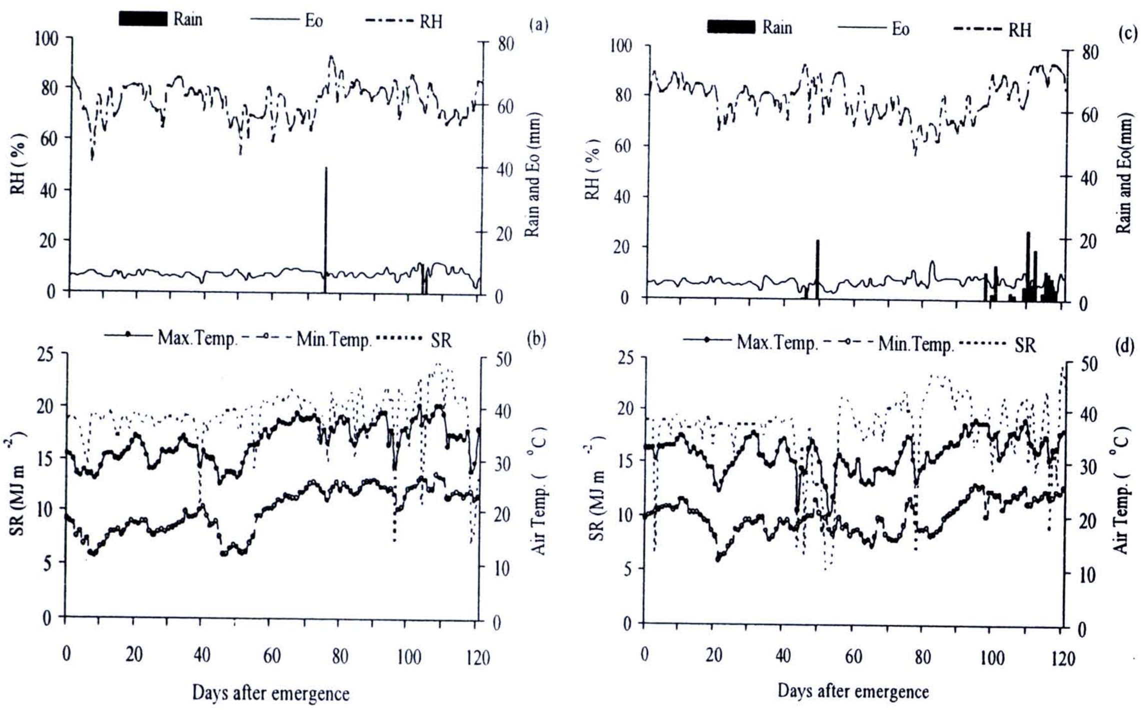

Fig. 1. Rain fall, evaporation (E_O), relative humidity (RH), maximum and minimum air temperature (Max and Min Temp.) and solar radiation (SR) in 2006/07 (a, b) and 2007/08 (c, d).

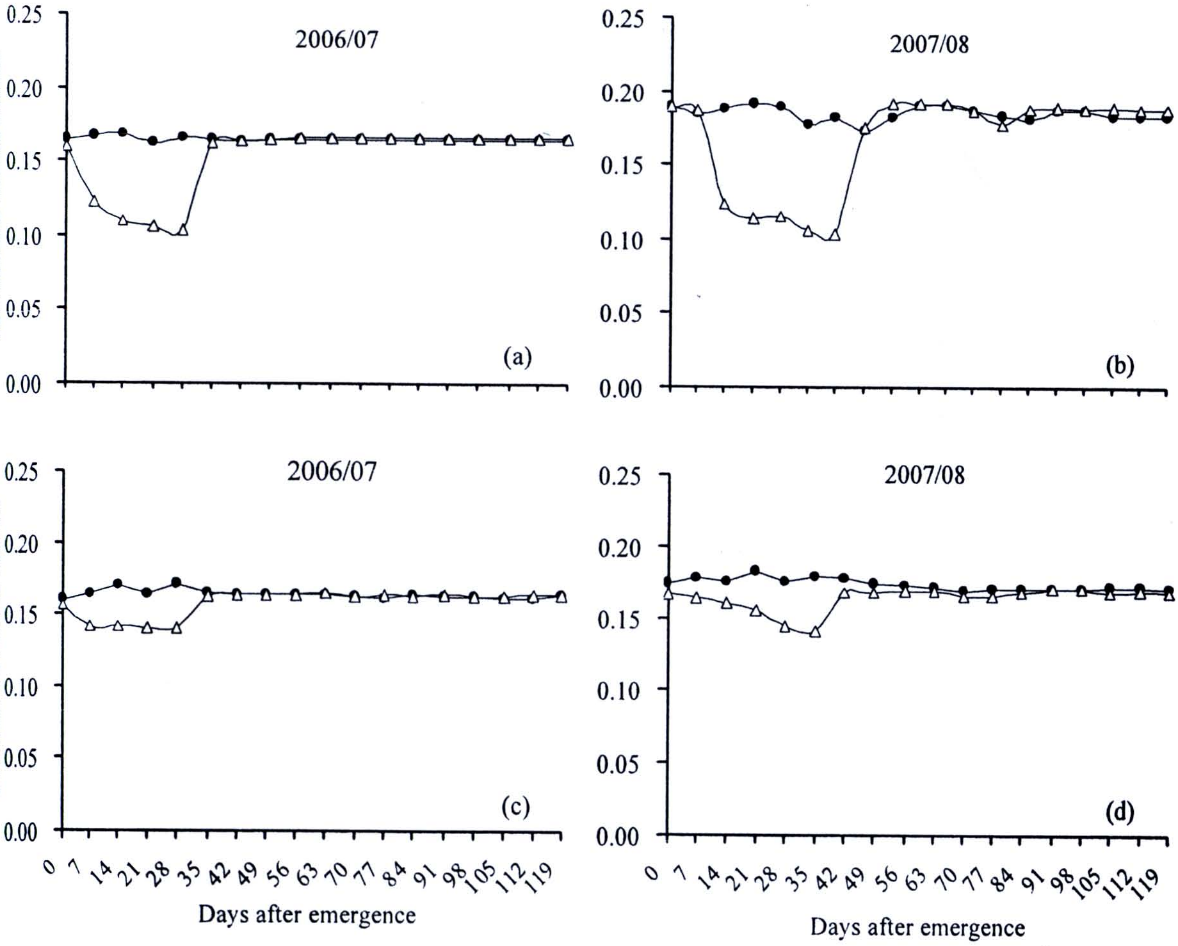

Fig. 2. Soil moisture volume fraction in two available soil water regimes [field capacity (FC), ●; and 1/3 available water (AW), Δ] at 30 cm (a, b) and 60 cm (c, d) of the soil level during the 2006/07 and 2007/08 dry seasons.

Table captions

Table 1 Analysis of variance of cross and cross product.

Table 2 Mean squares from the combined ANOVA for pod yield, biomass and drought tolerance index for biomass (DTI (BIO)) and pod yield (DTI (PY)) and harvest index (HI) at harvest, SPAD chlorophyll meter reading (SCMR) and specific leaf area (SLA) during water stress (40 DAE) and re-watering (60 DAE) under the irrigated and early season drought (ESD) of 90 lines in the dry season of 2006/07 and 2007/08.

Table 3 Means and range for pod yield, biomass, harvest index (HI), SLA and SCMR in 4 crosses and 4 parents of peanut under irrigated (IRR) and early season drought (ESD) conditions and DTI for pod yield (DTI (PY)) and biomass (DTI (BIO)).

Table 4 Estimates of heritability with standard error for biomass, drought tolerance index for biomass (DTI (BIO)), pod yield, drought tolerance index for pod yield (DTI (PY)), harvest index (HI), specific leaf area (SLA) and SPAD chlorophyll meter reading (SCMR) of 4 crosses of peanut under the early season drought (ESD) and irrigated conditions in the dry seasons of 2006/07 and 2007/08.

Table 5 Genotypic (r_G) correlation estimates among drought resistance traits for all 4 peanut crosses of 90 lines in the dry seasons of 2006/07 and 2007/08 (degree of freedom = 356).

Table 6 Genotypic (r_G) correlation estimates between drought resistance traits and agronomic traits for all 4 peanut crosses of 90 lines in the dry seasons of 2006/07 and 2007/08 (degree of freedom = 356).

Figure captions:

Fig. 1. Rain fall, evaporation (E_O), relative humidity (RH), maximum and minimum air temperature (Max and Min Temp.) and solar radiation (SR) in 2006/07 (a, b) and 2007/08 (c, d).

Fig. 2. Soil moisture volume fraction in two available soil water regimes [field capacity (FC), ●; and 1/3 available water (AW), Δ] at 30 cm (a, b) and 60 cm (c, d) of the soil level during the 2006/07 and 2007/08 dry seasons.

Rooting traits of peanut genotypes with different yield responses to pre-flowering drought stress

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Abstract

Water stress during the vegetative development normally is not detrimental and sometimes actually increases yield of peanut (*Arachis hypogaea* L.). Root growth might play an important role in response to early season drought in peanut and might result in an increase in yield. Information on the response of root characters of diverse peanut genotypes to these conditions will provide useful information for explaining mechanisms and improving peanut genotypes for exploiting positive interaction for pod yield under pre-flowering drought. The aim of this study, therefore, was to investigate the root dry weight and root length density of peanut genotypes with different yield responses to pre-flowering drought stress and their relationships with pod yield. Field experiments were conducted at the Field Crop Research Station of Khon Kaen University, Khon Kaen, Thailand during February to July, 2007 and during February to July, 2009. A split-plot experiment in a randomized complete block design was used. Two water management treatments were assigned as the main plots, i.e. field capacity and pre-flowering stress, and six peanut genotypes as the sub-plots. Total crop dry matter, root dry weight and root length density were recorded at 25 DAE, R5 and R7. Top dry weight and pod yield were measured at harvest and pod harvest index (PHI) was computed using the data on pod yield and biomass. Peanut genotypes were categorized into three groups based on their responses to drought for pod yield, e.g. increasing, decreasing and non-responsive groups. The genotypes of each group showed a differential response for root quantity and distribution. The increasing pod yield group had more root dry weight and root length density in the deeper soil layers during pre-flowering stress compared to the non-stress treatment. The Non-responsive group showed no root response under pre-flowering drought conditions compared to the non-stress treatment. A larger root system alone without

considering distribution may not contribute much to pod yield but a higher RLD at deeper layers may allow plants to mine more available water in the sub-soil. However, as yield is a complex trait, several mechanisms may be involved. The increasing pod yield group also had the ability to maintain a high PHI.

Keywords: root length density; root mass; drought tolerance; harvest index; early drought stress.

1. Introduction

Peanut is grown widely under rain-fed conditions in the semi-arid tropics. Drought is one of the major constraints, especially during the pod and seed formation stages, and it has shown to reduce pod yield by 56-85% (Nageswara Rao et al. 1989). However, water stress during the vegetative or early flowering stages is not detrimental and sometimes actually increases yield (Nageswara Rao et al. 1985, Nautiyal et al. 1999).

Most studies have reported on the response of physio-morphological characters of above ground plant components, but there is limited information for root characteristics. Nageswara Rao et al. (1985), and Nautiyal et al. (1999) found that vegetative growth, crop growth rate (CGR), pod growth rate (PGR) and reproductive development were associated with increased yield after pre-flowering drought stress. Awal and Ikeda (2002) reported that chlorophyll concentration, stomatal conductance, photosynthesis and relative growth rate (RGR) increased after re-watering. For root traits of one peanut genotype grown under water deficit during pre-flowering, Nageswara Rao et al. (1989) assumed that the promotion of root growth during drought stress was an important character contributing to the increased yield, but they did not measure root growth.

Drought resistance might be increased by improving the ability of the crop to extract water from the entire soil profile (Wright and Nageswara Rao, 1994). Rucker et al. (1995) found that some peanut genotypes that had a large root system (root dry weight) under non-stress conditions produced a higher yield under drought conditions and they suggested that these genotypes could possess drought avoidance traits. Rooting depth, root distribution and root length density (RLD) have been identified as drought avoidance traits (Passioura 1983, Turner 1986, Matsui and Singh 2003, Taiz and Zeiger 2006). However, Robertson et al. (1980) found no significant difference in rooting density of peanut cultivar Florunner under both irrigated and non-irrigated treatments. In contrast, Pandey et al. (1984) reported that drought increased root length density of a peanut genotype in the bottom part of the soil profile. Peanut genotypes that have a higher root length density in deeper soil layers have an enhanced drought tolerance, which can result in a higher pod yield and harvest index under long-term drought conditions (Songsri et al., 2008). Thus, root traits may be associated with differential yield responses to pre-flowering drought stress.

So far information about root response of peanut under pre-flowering drought conditions has been very limited in the literature. Awal and Ikeda (2002), who only studied one peanut genotype grown in containers, reported that drought significantly enhanced the root to shoot ratio which accelerated post-stress recovery. Meisner and Karnok (1992), who investigated root growth of a peanut genotype in a rhizotron chamber, found that the root growth rate was significantly reduced during stress from 20 to 50 days after planting (DAP) compared to non-stressed conditions under sufficient irrigation. After recovery, early drought-stressed peanut had more root growth than the non-stressed peanut of the control treatment. Most recently, Puangbut et al. (2009) reported differential pod yield responses to early season drought for six peanut genotypes under field conditions. However, the mechanisms underlying yield responses of these peanut genotypes have not been well understood because there was no information on rooting traits under these conditions. The results reported so far have been limited to experiments under greenhouse conditions and with a only a few peanut genotypes.

Roots could play an important role for yield increase in response to early season drought in peanut. Information on the responses of root characteristics of diverse peanut genotypes to pre-flowering drought under field conditions is still lacking and further investigations are necessary. Therefore, the goal of this study was to investigate the responses of root dry weight and root length density of peanut genotypes having different yield responses to pre-flowering drought stress and their relationships with pod yield.

2. Materials and Methods

2.1. Experimental details

Six peanut genotypes, e.g., KK 60-3, Tainan 9, Tifton-8, ICGV 98305, ICGV 98324 and ICGV 98330, differing in yield response to early season drought were selected from the study conducted by Puangbut et al. (2009). The genotypes ICGV 98305, ICGV 98324 and ICGV 98330 were provided by International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) and have been reported to be drought resistant. Tifton-8 is a drought resistant Virginia-type peanut developed by the United States Department of Agriculture (USDA; Coffelt et al., 1985). KK 60-3 and Tainan 9 are high yielding cultivars that have been released in Thailand. Puangbut et al.

(2009) found that the six genotypes could be separated into four different groups based on yield response to pre-flowering drought. The genotypes KK 60-3 and Tifton-8 were classified as having a highly positive response with significant yield increase. ICGV 98330 was classified as having a slight increase in pod yield. Tainan 9 and ICGV 98324 were classified as non-responsive, while ICGV 98305 was classified as having a reduction in pod yield.

Field experiments were conducted at the Field Crop Research Station of Khon Kaen University, Khon Kaen, Thailand (lat 16° 28′ N, long 102° 48′ E, 200 masl) from February to July, 2007 and from February to July, 2009. The experimental sites in the two seasons were adjacent fields. The soil type was a Yasothon series (Yt: fine-loamy; siliceous, isohypothermic, Oxic Paleustults). A split-plot experiment in a randomized complete block design with four replications was used. Two water management treatments were assigned as main plots and six peanut genotypes as sub-plots. The water management treatments were field capacity (FC) and pre-flowering drought (PFD). The FC treatment was maintained at FC from planting to harvest. For the PFD treatment, irrigation was withheld from 1 to 25 DAE. After this stress period, the PFD treatment was irrigated to FC, and the soil moisture content was maintained at FC until harvest. Rainout shelters were used to shield the PDF plots from rain. Plot size was 18.2 m^2 consisting of a seven-row plot with a 5.2 m row length. The spacing between rows was 50 cm and the spacing between plants was 20 cm for a plant density of 10 plants m^2.

2.2. Crop management

The soil was sub-soiled to break-up the hard pan that was present in the top 60 cm of the soil profile. Disc plowing was performed three times to prepare the individual plots for the experiment. Lime ($CaCO_3$) at a rate of 625 kg ha^{-1} was incorporated into the soil during soil preparation. Nitrogen fertilizer in the form of urea was applied at a rate of 23.4 kg N ha^{-1}, phosphorus fertilizer as triple superphosphate was applied at a rate of 24.7 kg P ha^{-1} and potassium fertilizer as potassium chloride was applied at a rate of 31.1 kg K ha^{-1} shortly prior to planting. Gypsum ($CaSO_4$) was applied to the soil surface at a rate of 312 kg ha^{-1} at 45 DAE. Seeds were treated with Captan (3a, 4, 7, 7a-tetrahydro-2-[(trichloromethyl)thio]-1H-isoindole-1, 3(2H)-dione) at a rate of 5 g kg^{-1} seed before planting. The seeds were over-planted, and the seedlings were thinned to one plant per hill at 7 days after emergence (DAE).

Carbofuran (2,3-dihydro-2,2-dimethyl benzofuran-7-ylmethylcarbamate 3 % granular) was applied at the pod setting stage. Pest 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 2.5 l ha^{-1}, methomyl [S-methyl-N-((methylcarbamoyl) oxy) thioacetimidate 40 % soluble powder] at 1.0 kg ha^{-1} and carboxin (5,6-dihydro-2-methyl-1,4-oxath-ine-3-carboxanilide 75 % wettable powder) at 1.68 kg ha^{-1}.

A drip-irrigation system was installed prior to planting and each plot was supplied with sufficient water to reach field capacity (FC) up to a depth of 60 cm. Soil moisture content was maintained uniformly at FC from planting to 50% emergence for the latest emerging line for all treatments. After emergence, the non-stressed treatment was maintained at FC until harvest. For the stressed treatment, irrigation was withheld starting at 1 DAE. As a result, the soil moisture content gradually decreased. After a stress period of 25 days, the stressed plots were irrigated to FC, and the soil moisture content was maintained at FC until harvest. A schematic presentation of soil moisture content for two water regimes is provided in Figure 1.

For treatment that had sufficient water, the soil water content was maintained uniformly at FC from planting until harvest, and moisture content was controlled with no more than 1% moisture change from FC using a gravimetric sample at 25, 30, and 50 DAE and at final harvest to check whether the water treatments were providing sufficient water (Table 1). The amount of water that was applied was calculated using crop water requirement as described by Doorenbos and Pruitt (1992). However, our previous study found that the amount of water that was applied based on this methodology could not maintain the soil moisture content at FC, resulting a soil moisture content that was less than FC. Therefore, the amount of water that was applied was based on crop water requirements using the Doorenbos and Pruitt (1992) methodology along with water loss from surface evaporation as described by Singh and Russel (1981). Thus, the amount of water that was supplied was calculated as the sum of crop water requirement and soil evaporation, and soil moisture content was determined using the gravimetric method.

Crop water requirement based Doorenbos and Pruitt (1992) was calculated as shown in Equation 1

$$ETcrop = ETo \times Kc, \qquad \text{Equation 1}$$

where, ETcrop = crop water requirement (mm/day), ETo = evapotranspiration of a reference crop under specified conditions calculated by pan evaporation method, Kc = the crop water requirement coefficient for peanut, which varied depending on growth stage (initial stage (1-15 DAE) Kc = 0.40, development stage (15-45 DAE) Kc = 0.70, mid-season (45-75 DAE) Kc = 0.95 and late season (75 DAE-harvest) Kc = 0.70).

Surface evaporation was calculated as (Singh and Russel, 1981):

$$Es = \beta \times (Eo/t) \qquad \text{Equation 2}$$

where, Es = soil evaporation (mm), ß = light transmission coefficient measured depending on crop cover, Eo = evaporation from class A pan (mm/day), t = days since the last irrigation (days).

2.3. Soil moisture content and meteorological conditions

Soil moisture content was determined using gravimetric method at planting, 25, 30, and 50 DAE and at final harvest at depths of 0-5 ,10-15, 25-30, 40-45, 55-60, 70-75 and 85-90 cm to verify that the irrigation treatments provided sufficient water. Soil moisture content was also measured with a neutron probe (Type I.H. II SER. N°NO 152, Ambe Diccot Instruments CO.Ltd., England). An aluminum access tube was installed between rows in each plot. Neutron probe readings were conducted at a depth of 30, 60 and 90 cm (30 cm intervals) at 5 day intervals throughout the course of the experiment. Rainfall, relative humidity (RH), evaporation (E_0), maximum and minimum temperature and solar radiation were recorded daily from sowing until harvest at a weather station located at a distance of 100 m from the experimental field.

2.4. Root traits

Root length density (RLD) was measured at 25 DAE, at first seed (R5; 53-59 DAE) and at physiological maturity (R7; 79-91 DAE) (Boote 1982) using an auger. The auger consisted of a coring tube (Welbank et al. 1974) with a diameter of 76 mm and a length of 1.15 m. The sampler was designed to reduce compaction in the inner tube by improving the cutting edge and reducing the tube thickness (Welbank et al. 1974, Ford et al. 2006). Two positions were collected, including the center of plant and the position between two row positions at a distance of 22.5 cm from each plant. Root samples were taken to a depth of 90 cm and separated into six layers consisting of 0-15, 15-30, 30-45, 45-60, 60-75 and 75-90 cm. Root samples of each layer were washed manually with tap water to remove soil from the roots. The root samples were then analyzed with the Winrhizo program (Winrhizo Pro (s) V. 2004a, Regent Instruments, Inc) to determine total root length per sample. RLD was calculated as the ratio between root length (cm) and soil volume (cm^3). RLD from the first (0–15 cm) and second (15–30 cm) layers were combined and defined as a single 0 to 30 cm layer or upper soil layer, while the RLD for the deeper layers (third to sixth) were combined to form a single 30 to 90 cm layer or lower soil layer. RLD was combined as upper and lower layers depending on tillage layer and differential soil moisture contents. The upper layer was defined as disc plowing layer, and soil moisture content of the two water regimes were clearly different at a soil depth of 30 cm (Figure 1). By contrast, the lower soil layer was defined as non tillage layer, and differences in soil moisture content between two treatments were small at 60 cm and soil moisture content was not significantly different at 90 cm (Figure 1).

Root dry weight was determined at 25 DAE, R5 (53-59 DAE) and R7 (79-91 DAE) using the monolith method for one plant per plot. The size of monolith was 50 x 20 cm with a depth of 50 cm. The roots were removed from the monolith soil sample using the same method described previously for the core sample. The root samples were oven-dried at 80 C° for 48 hours or until constant weight and root dry weight was determined.

2.5. Biomass, pod yield and pod harvest index (PHI)

Biomass samples, including shoots, roots (not available at final harvest) and pods (available at R7 and harvest only), were obtained at 25 DAE, R5 (53-59 DAE) and R7 (79-91 DAE) and harvest (112-132 DAE). Five plants in each plot were harvested. The sample was oven-dried at 80 ^{o}C for 48 h or until constant weight and dry weight was measured. Biomass was then calculated and used in determining the total biomass per unit land area. At final harvest, a total area of 7.5 m^2 was harvested from each plot. The pods were removed from the plants and air-dried to approximately 8% moisture content and pod dry weight determined. Shoot fresh weight from the harvested plants was determined, oven-dried, and weighed. PHI was calculated as pod dry weight per unit total biomass at harvest.

The drought tolerance index (DTI) was computed for pod yield, biomass, PHI, root dry weight and root/shoot ratio by comparing values under stress treatment to values for the field capacity treatment as suggested by Nautiyal et al. (2002) (more than 1= increased, less than 1 = decreased).

DTI = Data of stress treatment / Data of non stress treatment.

2.7. Statistical analysis

The statistical analysis was conducted using MSTAT-C package (Bricker, 1989). The measured data were subjected to analysis of variance according to a split plot design. Error variances for the two years were tested for homogeneity using the Bartlett's test (Gomez and Gomez 1984), and then data for each year were analyzed separately because the G x E interaction for all variables was significantly (data not shown). Therefore, the results of each variable are shown for each year. The comparison between two means of each genotype under two water regimes for all of parameters was done based on the Least Significant Difference (LSD) test (Gomez and Gomez 1984).

3. Results and Discussion

3.1. Meteorological conditions and soil moisture content

The first experiment was conducted from February to June 2007. The average air daily temperature ranged from 25.2 to 34.9°C during the growing season (Figure 2). Total rainfall during the drought-stress period was 15.1 mm, while total rainfall after the drought-stress period was 428.2 mm. The second experiment was conducted from February to June 2009. The average air daily temperature ranged from 24.9 to 34.5°C during the growing season (Figure 2). Total rainfall during the drought-stress period was 6.2 mm, while total rainfall after the drought-stress period was 414.9 mm. Due to the rain both experiments required the use of the rainout shelter during the water stress period. While the rainfall was large after the pre-flowering drought stress, this occurred after 25 DAE and, therefore, did not affect growth during the drought-stress period.

Soil moisture content of the two water regimes during both seasons was clearly different at a soil depth of 30 cm (Figure 1). The differences were small at 60 cm and soil moisture content was not significantly different at 90 cm. The differences in soil moisture content between the two water regimes decreased with the depth of the soil profile. The soil moisture content measurements also confirmed adequate control of the irrigation applications.

3.2. The responses to pre-flowering drought conditions for yield and pod harvest index

The peanut genotypes were categorized into three groups based on the responses to pre-flowering drought for pod yield using the comparison between two means of water regime treatments of each genotype based on the LSD at $p<0.05$. The drought tolerance index (DTI) is the ratio of pod yield for pre-flowering drought treatment to pod yield for the non-stressed treatment, and this was used to represent the response.

In both seasons, ICGV 98305 was classified as increasing genotype with a DTI of 1.53 for the first season and a DTI of 1.36 for the second season (Table 2). There was significant increase of pod yield under PFD conditions of ICGV 98305 comparing to sufficient water conditions. Tainan 9 and ICGV 98324 showed an increase in pod yield under pre-flowering drought conditions. However, pod yield under PFD conditions compared to non-water stress conditions for Tainan 9 was only significant for the first season and DTI of this genotype as 1.41. In the second season, Tainan 9 did not have a significantly higher ratio (DTI= 1.16). For ICGV 98324, the first season was not significant (DTI=1.23). However, there was significant increase in pod yield under pre-flowering drought conditions in the second season (DTI=1.57). ICGV 98330 was classified as decreasing in the first season (DTI = 0.76). However, the second season was not significant (DTI = 0.84), but it seemed likely that the response of this genotype could be classified as decreasing. KK 60-3 and Tifton-8 were non responsive genotypes (DTI 1^{st} season = 1.09 and 0.85 respectively and 2^{nd} season = 0.98 and 1.26 respectively) (Table 2).

The responses of pod yield between Puangbut et al. (2009) and this study were different for the peanut genotypes that were selected based on the Puangbut et al.

(2009) study. This could be due to the differences of the drought stress treatments. In Puangbut et al. (2009), irrigation was withheld from emergence onward until soil moisture content was reduced to 1/3 available water (1/3 AW; soil moisture content for AW was the values between FC and permanent wilting point that were proportional to soil moisture at FC) and the soil moisture was maintained at this level until 40 DAE. In this experiment, soil moisture was allowed to decline from emergence until 25 DAE. As no water was supplied during the drought period, the soil moisture content in this study was lower than 1/3 AW. The soil moisture content at 1/3 AW in these experiments was 6.63% in 2007 and 7.06% in 2009. On the most stressed date soil moisture content at the 0 to 30 cm soil depth was 6.06 and 6.75% in 2007 and 2009, respectively (Table 1). After re-watering to FC the soil moisture content of the PFD treatment were 11.14 % and 11.55 % in 2007 and 2009 by 30 DAE, respectively, and 10.36 % and 10.55 % in 2007 and 2009 by 50 DAE, respectively, (Table 1) The soil moisture content percentages measured after drought period showed values close to field capacity, e.g. 10.44 % and 11.26 % (Table 1). Drought in this study was similar to a naturally occurring drought in that it was rather severe and over a shorter period than the experiment of Puangbut et al. (2009).

Although some peanut genotypes had increased pod yield after early drought conditions, this was not true for all the genotypes in this study. Peanut genotypes have different yield response to pre-flowering stress. A similar drought tolerance response of ICGV 98305 was observed by Wunna et al. (2009) who showed that only the genotype ICGV 98305 out of eleven genotypes gave a higher pod yield after early drought stress. Songsri et al. (2008) studied the responses of reproductive traits in peanut genotypes to long-period drought stress and found that the genotype ICGV 98305 showed a small reduction in pod yield reduction under drought stress.

Therefore this genotype has good maintenance of pod productivity when subject to drought stress.

In this study, at growth stage 25 DAE, R5 and R7 when growth analysis samples were collected, there was no genotype which showed consistent responses of biomass, to pre-flowering drought (Table 3). The short period of drought in this study might not have affected total biomass, but only affected partitioning to root growth. A similar result was presented by Nautiyal et al. (1999). Meisner and Karnok (1992) reported that water stress during vegetative phase did not significantly affect leaf and stem dry weight.

At final harvest in both seasons, ICGV 98305 was the only genotype that showed significantly higher pod harvest index (PHI) under pre-flowering drought compared to FC (Table 2). A similar higher PHI under a long period of drought stress conditions has also previously been reported for ICGV 98305 (Songsri et al., 2008). Wunna et al. (2009) found that ICGV 98305 had a higher PHI after early drought stress. This confirms the consistency of performance of this genotype under drought stress conditions. The harvest index has been identified as a drought resistance trait in peanut (Nigam et al. 2003, 2005), and the ability to partition dry matter into harvestable yield under limited water supply is an important trait for drought tolerant genotypes (Chapman et al., 1993).

3.3. The responses of rooting traits to pre-flowering drought conditions

The genotypes of the different groups had differential responses for root dry weight and RLD. At 25 DAE, most of the genotypes under PFD had a higher root dry weight than the well-irrigated counterparts. However, ICGV 98305, Tainan 9 and KK 60-3 showed a significantly higher root mass under PFD treatment than under well-irrigated treatment during the first season (Table 4). For the second season, at 25 DAE, ICGV 98305 and ICGV 98324 had a higher root dry weight under pre-flowering drought conditions. Those genotypes that were classified as having an increase in pod yield in the PFD treatment compared to the non-stressed treatment were generally the same as the genotypes that showed a response of root dry weight by the end of the PFD period. The significant differences in root dry weight existed at 25 DAE only as these significant differences did not persist for the root dry weights observed at R5 and R7 (*data not shown*).

Only the genotype ICGV 98305 had a higher root/shoot ratio under pre-flowering stress conditions when compared to normal conditions at 25 DAE, and its DTI values for root/shoot ratio were 1.80 and 1.69 for the first and second season, respectively (Table 4). For the genotypes ICGV 98324, ICGV 98330, Tifton-8, Tainan 9 and KK 60-3, the root/shoot ratios of the stressed treatment were not statistically different from the root/shoot ratios under well-watered conditions. A larger root/shoot ratio of peanut in response to drought stress conditions results from partitioning a larger proportion of assimilates to roots during this drought-stressed period compared to non-stressed conditions. Peanut appears to adapt to drought conditions by increasing root length to mine more available water (Alycmeny, 1997; Mayaki et al., 1976). Similar observations have also been reported in rice (Nemoto et al., 1998; Kondo et al., 2003), chickpea (Kashiwagi et al. 2006), cowpea (Matsui and Singh 2003), and soybean (Hoogenboom et al., 1987; 1988). The differences among peanut genotypes in response to early season drought in this study provide useful information, and suggest the value of selecting peanut genotypes with high root/shoot ratio for drought resistance breeding.

The value of a large root system related to pod yield has been well demonstrated in peanut. Rucker et al. (1995) found that some peanut genotypes with large root system (root dry weight) under non-stress conditions gave higher yield under drought conditions. Moreover, root dry weight was highly correlated to shoot dry weight, leaf area and number of leaves (Ketring, 1984). However, a deeper root system that contributes to maintaining yield under drought stress conditions has not been clearly demonstrated. A larger root system alone may not contribute much to pod yield if the increase in roots is not distributed into wetter or deeper soil. The response of RLD if into deeper soil layers may allow plants to be able to mine more available water in the sub-soil (Songsri et al., 2008).

At 25 DAE, the root distribution sampled at the center of the plant position was not significantly different between genotypes receiving different water treatments (*data not shown*). In Arabidopsis, drought stress has relatively little effect on the growth of primary roots to mild drought stress (Xiong et al., 2006). In this study, pre-flowering drought might have slightly affected for the RLD distribution of primary root. Therefore, RLD at the center of the plant position did not occur different between the two water management treatments. On the other hand, there were significant differences between the two water management treatments in root distribution sampled at the inter-row position (Table 4). The responses for root distribution sampled at the inter-row position were quite similar to those for root dry weight. For RLD of the upper soil layer (0-30 cm), only ICGV 98305 had a higher RLD under the water stress treatment than normal conditions. For the deeper soil layer (30-90 cm), ICGV 98305 and Tainan 9 had a significantly higher RLD under drought than under well-watered conditions in season 1 (DTI = 4.09 and 3.12 respectively). In season 2, ICGV 98305, ICGV 98324 and KK 60-3 had a significantly higher RLD under pre-flowering drought conditions (DTI = 7.24, 9.02 and 3.47 respectively). RLD at inter-row position and in deeper soil layer may be involved with differential yield responses to pre-flowering drought stress. Other studies have shown that drought increased root length density in the lower soil profile of peanut (Pandey et al., 1984). The peanut genotypes that had a higher root length density in the deeper soil layers potentially have an enhanced drought tolerance and this could aid peanut genotypes to obtain higher pod yield and harvest index under long-term drought conditions (Songsri et al., 2008).

The RLD differences occurred only at 25 DAE, and the differences did not persist up to R5 and R7. Based on this finding, greater RLD at inter-row position and in deeper soil layer is one important factor which may increase pod yield under pre-flowering drought stress. Since the effect is relatively minor in terms of total dry matter shift to roots in the deeper layer, the mechanism may be as much as modifying plant growth regulator regulation of partitioning as it is in the uptake of water from the deeper soil layers (Songsri et al., 2008).

In this study, average RLD at the 0-90 cm soil profile might be one factor affecting harvested yield even though some of the soil depth layers did not show significant differences in RLD under sufficient and stressed water treatment. However, there was a tendency of greater average RLD at the 0-90 cm soil profile in the PFD increasing pod yield group, and this remained high even after re-watering (Figure 3). On the other hand, the decreasing yield genotype such as ICGV 98330, had lower average RLD at R7 under PFD than in the sufficient irrigation treatment (Figure 3). In chickpea, Kashiwagi et al. (2006) studied variability of root length density and reported that average RLD at the 0-60 cm soil profile was highly correlated with seed yield under sufficient water conditions. However, pod yield is a complex trait resulting from the contribution of many genetic characteristics, which may influence PHI (Chapman et al. 1993, Songsri et al. 2008, Wunna et al. 2009), vegetative growth, reproductive development (Nageswara Rao et al. 1985, Nautiyal et al. 1999), and transpiration efficiency (Puangbut et al, 2009). Therefore rooting is only one important trait contributing to pod yield.

4. Conclusions

In summary, peanut genotypes were classified into three groups based on the pod yield responses to pre-flowering drought, e.g. increasing, decreasing and non responsive yield groups. The genotypes in different groups had differential responses for root quantity and distribution. In the group with increased pod yield, such as ICGV 98305, root dry weight and root length density were greater in deeper soil layer in pre-flowering stress compare with non-stress treatment. In the genotype with decreased pod yield, ICGV 98330 had small increase in root dry weight and root length density at deeper soil layer. Larger root system alone may not contribute much to pod yield if the large root portion is not distributed into moist soil. The response of RLD might allow plants to be able to mine more available water in sub-soil. However, PHI is also an important outcome for drought resistance. As yield is a complex result of many mechanisms and traits, root dry weight and RLD may only contribute to PHI and pod yield. The knowledge of responses of root traits and relationships with pod yield will be useful for breeding of peanut for pre-flowering drought environment.

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Figure Legends and Table Captions

Figure 1 Volumetric soil moisture (fraction) in two water regimes as well-watered (FC;●) and pre-flowering drought (PFD;○) the experiments were conducted at the Field Crop Research Station of Khon Kaen University, Thailand during February-June 2007 (1st season) at 30 cm (a1), 60 cm (a2) and 90 cm (a3) of the soil level and repeated during February-June 2009 (2nd season) at 30 cm (b1), 60 cm (b2) and 90 cm (b3) of the soil level.

Figure 2 Rainfall, humidity (RH), evaporation (E0), maximum (Tmax) and minimum (Tmin) temperature and solar radiation during February-June 2007(a,b) and 2009 (c,d) at the meteorological station, Khon Kaen University, Thailand.

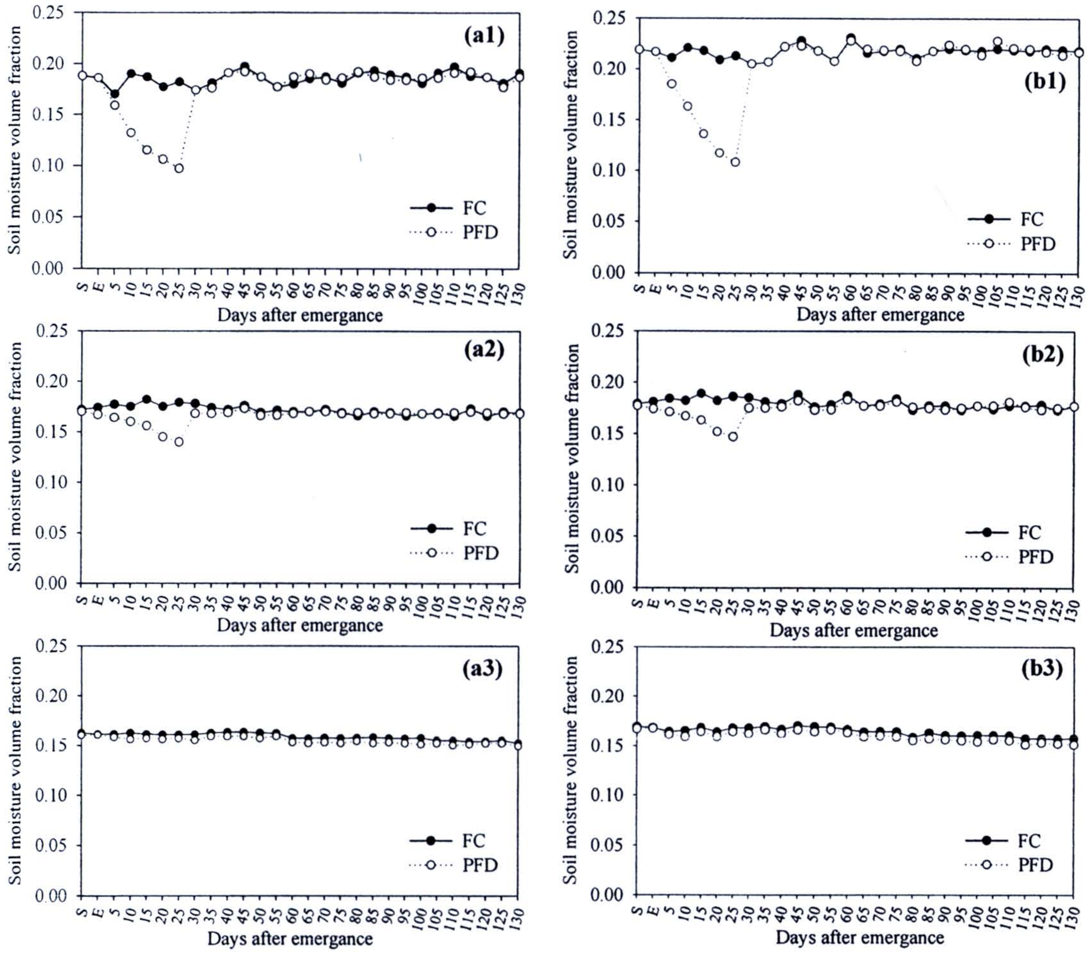
Figure 3 Average root length density (RLD) at the 0-90 cm soil profile of some peanut genotypes, measured over time, at 25 day after emergence (DAE), first seed (R5; 53-59 DAE) and beginning maturity (R7; 79-91 DAE) under well-watered (FC;●) and pre-flowering drought (PFD;○) the experiments were conducted at the Field Crop Research Station of Khon Kaen University, Thailand during February-June 2007 (season 1) and in 2009 (season 2).

Table 1 Soil moisture percentage (%) at 25 day after emergence (DAE), 30 DAE, 50 DAE and harvest under well-watered (FC) and pre-flowering drought (PFD) the experiments were conducted at the Field Crop Research Station of Khon Kaen University, Thailand during February-June 2007 (season 1) and in 2009 (season 2).

Table 2 Pod dry weight (kg/ha) and pod harvest index (PHI) of six peanut genotypes grown under well-watered (FC) and pre-flowering drought (PFD) the experiments were conducted at the Field Crop Research Station of Khon Kaen University, Thailand during February-June 2007 (season 1) and in 2009 (season 2).

Table 3 Biomass (kg/ha) of six peanut genotypes grown under well-watered (FC) and pre-flowering drought (PFD), measured at 25 day after emergence (DAE), first seed (R5; 53-59 DAE) and beginning maturity (R7; 79-91 DAE) at the Field Crop Research Station of Khon Kaen University, Thailand during February-June 2007 (season 1) and in 2009 (season 2).

Table 4 Root dry weight (RDW), root shoot ratio (R/S ratio) and root length density (RLD) in deeper soil layer (30-90 cm) at inter-row position at 25 day after emergence (DAE) of six peanut genotypes grown under well-watered (FC) and pre-flowering drought (PFD) at the Field Crop Research Station of Khon Kaen University, Thailand during February-June 2007 (season 1) and in 2009 (season 2).



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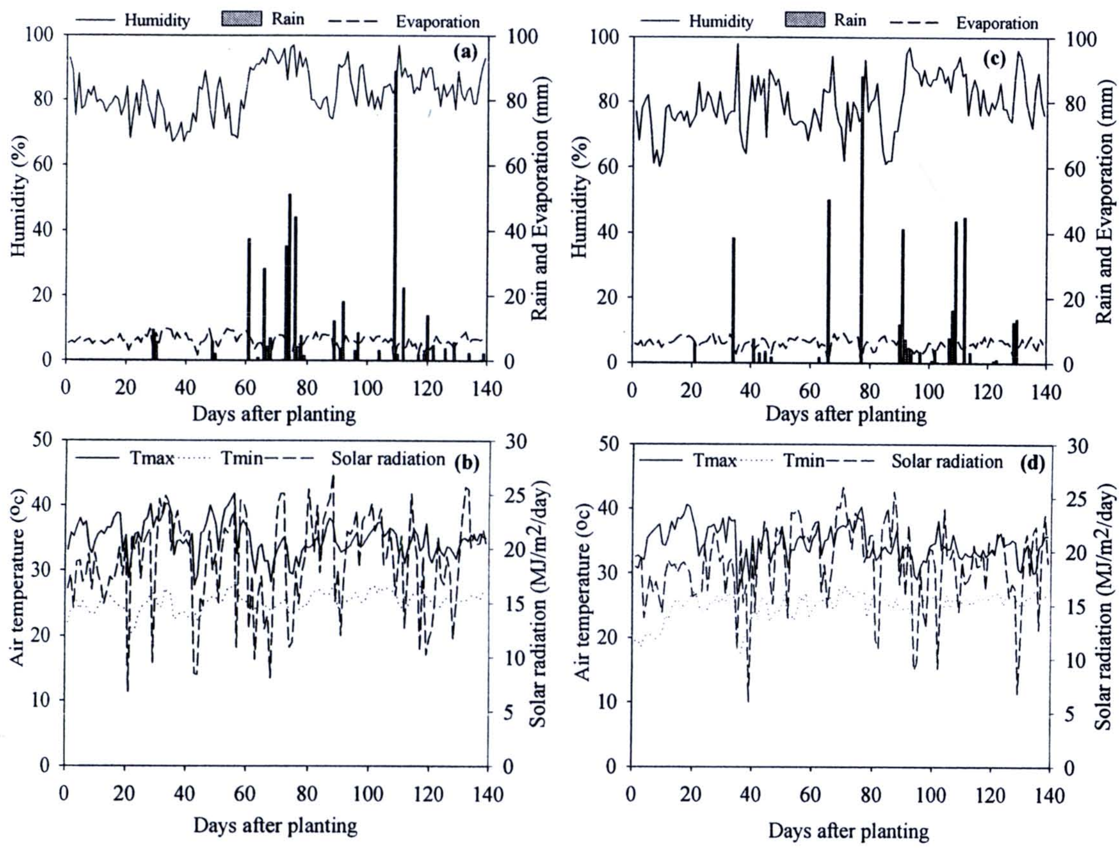

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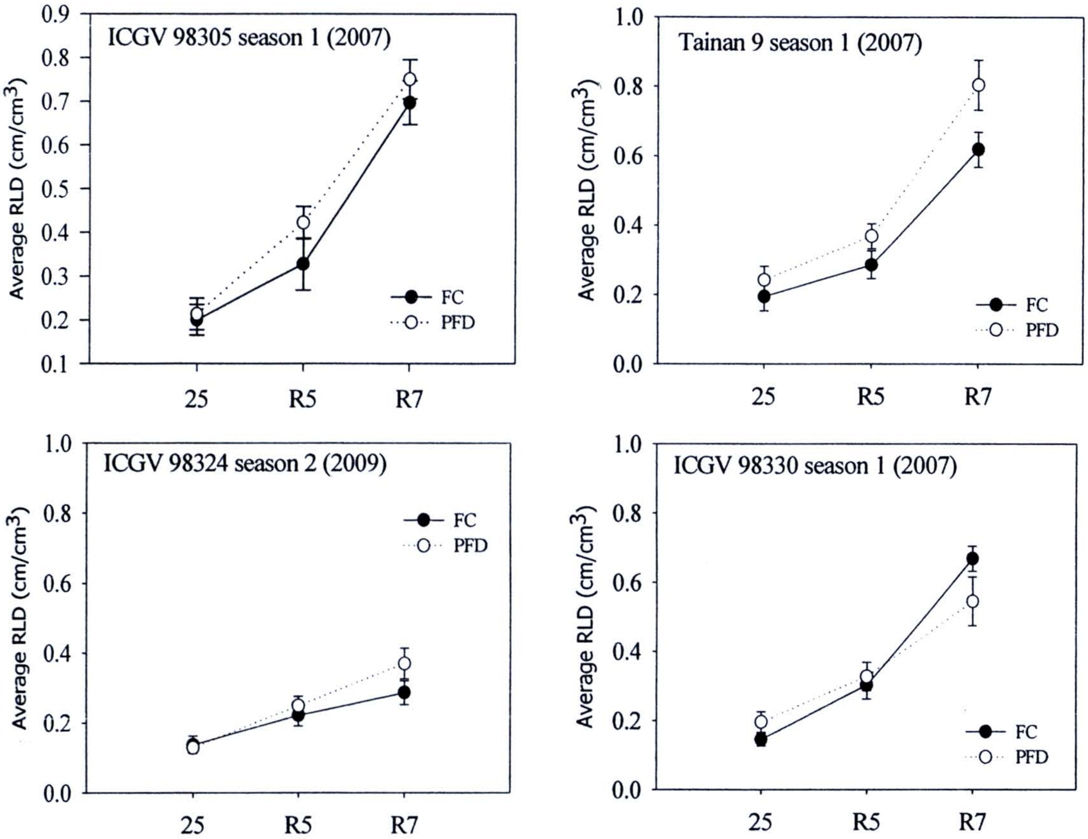

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Treatments	**Seasons**	**Soil moisture percentage (%)**			
		25 DAE	**30 DAE**	**50 DAE**	**Harvest**
FC	Season 1	10.67	10.93	10.87	10.33
	Season 2	10.53	11.62	11.27	11.80
PFD	Season 1	6.06	11.14	10.36	10.74
	Season 2	6.75	11.55	10.55	10.84

FC level of season 1 = 10.44% and season 2 = 11.26% using pressure plate method.

Table 2 Pod dry weight (kg/ha) and pod harvest index (PHI) of six peanut genotypes grown under well-watered (FC) and pre-flowering drought (PFD) the experiments were conducted at the Field Crop Research Station of Khon Kaen University, Thailand during February-June 2007 (season 1) and in 2009 (season 2).

Cultivar	Season	Water regime	Pod dry weight (kg/ha)	PHI
ICGV 98305	season 1	FC	1086b	0.103b
		PFD	1659a	0.161a
		DTI	1.53	1.56
	season 2	FC	1089b	0.114b
		PFD	1487a	0.166a
		DTI	1.36	1.45
Tainan 9	season 1	FC	1635b	0.171
		PFD	2308a	0.226
		DTI	1.41	1.32
	season 2	FC	1626	0.207
		PFD	1886	0.233
		DTI	1.16	1.13
ICGV 98324	season 1	FC	1739	0.181
		PFD	2145	0.221
		DTI	1.23	1.22
	season 2	FC	1141b	0.157
		PFD	1795a	0.197
		DTI	1.57	1.26
KK 60-3	season 1	FC	2321	0.176
		PFD	2528	0.193
		DTI	1.09	1.09
	season 2	FC	1549	0.194
		PFD	1521	0.176
		DTI	0.98	0.91
Tifton-8	season 1	FC	1567	0.152
		PFD	1338	0.118
		DTI	0.85	0.77
	season 2	FC	879	0.096
		PFD	1106	0.098
		DTI	1.26	1.02
ICGV 98330	season 1	FC	2060a	0.197
		PFD	1574b	0.159
		DTI	0.76	0.81
	season 2	FC	1515	0.196
		PFD	1272	0.158
		DTI	0.84	0.81

Different letters adjacent to data of a cultivar within a season in the same column show significance at $P < 0.05$ by LSD

DTI = drought tolerance index (stress/FC; more than 1= increased, less than 1 = decreased)

Table 3 Biomass (kg/ha) of six peanut genotypes grown under well-watered (FC) and pre-flowering drought (PFD), measured at 25 day after emergence (DAE), first seed (R5; 53-59 DAE) and beginning maturity (R7; 79-91 DAE) at the Field Crop Research Station of Khon Kaen University, Thailand during February-June 2007 (season 1) and in 2009 (season 2).

Cultivar	**Season**	**Water regime**	**Total crop biomass (kg/ha)**		
			25 DAE	**R5**	**R7**
ICGV 98305	season 1	FC	578	2622	7264
		PFD	687	3263	7041
		DTI	1.19	1.24	0.95
	season 2	FC	559	2316	4345
		PFD	523	1718	3208
		DTI	0.94	0.74	0.74
Tainan 9	season 1	FC	614b	2770	7133
		PFD	794a	3393	7212
		DTI	1.29	1.23	1.02
	season 2	FC	652	3196a	8493a
		PFD	591	1947b	6594b
		DTI	0.91	0.61	0.64
ICGV 98324	season 1	FC	489b	2722	7796
		PFD	648a	2878	7119
		DTI	1.33	1.06	0.85
	season 2	FC	463	2257a	7302
		PFD	401	1013b	6006
		DTI	0.87	0.45	0.68
KK 60-3	season 1	FC	642	3201	8853a
		PFD	744	3398	6774b
		DTI	1.16	1.06	0.63
	season 2	FC	588	2327	7402
		PFD	545	1810	6652
		DTI	0.93	0.78	0.82
Tifton-8	season 1	FC	661	3690	7849
		PFD	712	3426	7058
		DTI	1.08	0.93	0.83
	season 2	FC	544	2279	6950
		PFD	533	2374	7645
		DTI	0.98	1.04	1.19
ICGV 98330	season 1	FC	558b	2610b	7103
		PFD	734a	3799a	7630
		DTI	1.31	1.46	1.14
	season 2	FC	435	1336	6165
		PFD	575	2390	7321
		DTI	1.32	1.79	1.39

Different letters adjacent to data of a cultivar within a season in the same column show significance at $P < 0.05$ by LSD

DTI = drought tolerance index (stress/FC; more than 1= increased, less than 1 = decreased)

Table 4 Root dry weight (RDW), root shoot ratio (R/S ratio) and root length density (RLD) in deeper soil layer (30-90 cm) at inter-row position at 25 day after emergence (DAE) of six peanut genotypes grown under well-watered (FC) and pre-flowering drought (PFD) at the Field Crop Research Station of Khon Kaen University, Thailand during February-June 2007 (season 1) and in 2009 (season 2)

Cultivar	Season	Water regime	RDW(kg/ha)	R/S ratio	RLD (cm/cm^3) in 30-90 cm
ICGV 98305	season 1	FC	64b	0.130b	0.024b
		PFD	135a	0.234a	0.098a
		DTI	2.12	1.80	4.09
	season 2	FC	92b	0.175b	0.026b
		PFD	103a	0.296a	0.195a
		DTI	1.13	1.69	7.24
Tainan 9	season 1	FC	83b	0.103	0.023b
		PFD	132a	0.168	0.074a
		DTI	1.58	1.63	3.12
	season 2	FC	100	0.197	0.011
		PFD	102	0.268	0.036
		DTI	1.02	1.36	3.34
ICGV 98324	season 1	FC	85	0.208	0.026
		PFD	79	0.138	0.062
		DTI	0.93	0.66	2.40
	season 2	FC	92b	0.272	0.019b
		PFD	100a	0.298	0.179a
		DTI	1.10	1.10	9.02
KK 60-3	season 1	FC	59b	0.159	0.009
		PFD	108a	0.199	0.03
		DTI	1.83	1.25	3.18
	season 2	FC	100	0.153	0.074b
		PFD	99	0.217	0.258a
		DTI	0.99	1.42	3.47
Tifton-8	season 1	FC	99	0.174	0.057
		PFD	127	0.215	0.078
		DTI	1.29	1.23	1.38
	season 2	FC	95	0.238	0.034
		PFD	100	0.266	0.102
		DTI	1.06	1.12	3.02
ICGV 98330	season 1	FC	71	0.147	0.061
		PFD	91	0.140	0.048
		DTI	1.27	0.95	0.784
	season 2	FC	93	0.253	0.020
		PFD	97	0.209	0.041
		DTI	1.05	0.83	2.01

Different letters adjacent to data of a cultivar within a season in the same column show significance at $P < 0.05$ by LSD

DTI = drought tolerance index (stress/FC; more than 1= increased, less than 1 = decreased)

SSR-based detection of genetic variability among peanut lines *(Arachis hypogaea* L.*)* different in Specific Leaf Weight and Relative Water Content

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Abstract

The objective of this study was undertaken to identify superior parental lines different in SLW and RWC and genetics level by using SSR markers. Four peanut genotypes and two water regimes (FC and 1/3 available water; 1/3 AW) were arranged in a split plot design with six replications. The data were recorded for specific leaf weight (SLW) and relative water content (RWC). Also four peanut genotypes were used to detected genetic diversity using 265 genomic SSR markers. Drought increased SLW and reduced RWC. ICGV 39353 had the highest SLW under well water and drought conditions. ICGV 98324 could maintain the highest RWC, whereas KK 4 seem to be the most sensitive to drought as it has the lowest SLW and RWC. SSR markers could effective to detect the narrow genetics based of cultivated peanut. There were 182 markers showed clearly appeared on PAGE. Out of 182 markers there were 89 markers could detect polymorphism among peanut genotypes (48.9%). The numbers of alleles were ranged from 1 – 6 with a mean of 2.7 alleles per locus. The polymorphic information content (PIC) values were varied from 038 – 0.78 with a mean of 0.5. The genetics relationship among peanut genotypes was estimated. KK 4 was clustered distinct from the others genotypes, whereas ICGV 98324 and ICGV 98303 were grouped in the same cluster furthest from the KK 4. The result from this study should be useful as a source of variation for development of mapping population for drought tolerance in peanut breeding program.

Key words: drought, water regime, polymorphism, genetic relationship

Introduction

Most peanut production areas are in the semi-arid tropics, which were affected by drought stress because unpredictable rainfall and rain distribution (Wright and Nageswara Rao, 1994). Drought stress can occurred at any time during crop growth, causing severe yield loss and poor seed quality. Therefore, attempts have been taken to improve peanut cultivars with tolerance to drought stress (Branch and Kvien, 1992). The conventional approach of breeding for drought tolerances has been based on pod yield. However, selection for pod yield is difficult because of large effect of genotype x environment interaction. The physiological traits associated with drought tolerance with high heritability and less affected of environmental variation have been suggested to determine the superior drought tolerant genotypes (Songsri et al., 2008). Specific leaf weight (SLW) and relative water content (RWC) are physiological parameters related to drought tolerance (Nigam et al., 2008; Nautiyal et al., 1995). Peanut genotypes with high SLW had higher Transpiration efficiency (Brown et al., 1996), this trait had low genotype x environment interaction and high heritability (Songsri et al., 2008). SLA (closely associated with SLW) also has negative relationship with stomatal conductance, Riburose – 1,5-bisphosphate Carboxylase - oxygenase (Rubisco) enzyme and leaves and carbon exchange rate (CEC). Under water limited conditions, peanut genotypes with low SLA could maintain higher RWC and normal growth (Nautiyal et al., 2002). Thus, it is possible to use SLA to evaluate drought tolerance in peanut (Vasanthi et al., 2006; Upadhyaya, 2005).

DNA markers have been applied in many plants breeding program. The progress of selection should be more rapid than the conventional methods because it can be conducted in early generation of segregating population with more accuracy (Stalker and Mozingo, 2001). The methods for identifying markers and traits associations are based on genotypic and phenotypic data from specific crossed population that shows a difference in the target traits and genetics level. The cultivated peanut has limited genetic variability. Based on many marker types studied such as isozyme, Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphic DNA (RFLP), Amplified Fragment Length Polymorphic (AFLP) could not detect the polymorphism or had very low polymorphic among cultivated peanuts because of narrow genetic base. (Halward et al., 1991; Halward et al., 1992; Singh et al, 1993; Lanham and Fenneil, 1992; Lack and Stalker, 1993; Gracia et al., 1995; Stalker et al., 1995 ; Kochert et al., 1996; He et al., 1997; He et al., 2001; Gimenes et al., 2002). Hopkin et al. (1999) first developed Simple Sequence Repeats (SSRs) technique for peanut, and this technique was further used successfully to detect polymorphism in cultivated peanuts (Krishna et al., 2004; Ferguson et al., 2004; Moretzsohn et al., 2004). These markers are small arrays of tandem arranged bases (one to six) spread throughout the genomes and are abundant, informative, and co-dominant in nature. Recently SSR markers have been recognized as useful tools in plant breeding program such as genetic diversity analysis (Cuc et al., 2008, He et al., 2005, Jiang et al., 2007 and Mace et al., 2006), germplasm management (Barkley et al., 2007), genome mapping, QTL analysis and applicable for marker assisted selection . (Chenault et al., 2009, Ferguson et al., 2004, Varshney et al., 2008 and Yan bin et al., 2008).

The present study was undertaken to identify superior parental lines different in SLW and RWC and genetics level by using SSR markers. The information should be useful as a source of variation for development of mapping population for drought tolerance in peanut breeding program.

MATERIALS AND METHOD

Plant materials

Four peanut genotypes consisting of three drought tolerance lines from ICRISAT (ICGV 98324, ICGV 98353 and ICGV 98303) and one genotype (KK 4) commonly grown in Thailand, a low SLW and RWC cultivar (Boontang et al., inpress; Akkasaeng et al., 2007) was used to determine the genotypic variability in SLW and RWC under well-water and water limited conditions.

Physiological measurement

The pot experiments were conducted under field conditions at the Field Crop Research Station of Khon Kaen University located in Khon Kaen province, Thailand (latitude 16° 28′ N, longitude 102° 48′ E, 200 m above mean sea level; AMSL) during November 2006 to April 2007. Using 2 x 4 factorial in randomized complete block design (RCBD) with 6 replications. Two soil moisture levels at field capacity (FC) (10.28%) and 1/3 available water (AW) (5.33%) were assigned as factor A and 4 peanut genotypes were assigned as factor B.

The soil on the experimental site pertains to the Yasothon series (loamy sand, Ocix Paleustults). The proportion of sand, silt and clay in the soil were 71.25%, 20% and 8.75%, respectively. A sandy loam soil with pH 5.58, 0.47 % organic matter and 0.0255% total nitrogen (N). Available phosphorus (P) was 7 ppm (Bray II & Molybdenum-blue method) and extractable potassium (K) and calcium (Ca) were 23.5 and 216.5 ppm respectively.

The plants were grown in column pot having a diameter 25 cm and height 70 cm. Each pot was filled to 10 cm from the top with 43.6 kg to created uniform bulk density. three holes were made at the height level 15, 30 and 45 cm from the bottom of each pot to facilitate watering and distribution of water. 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} seed before planting, and treated with ethrel 48% at the rate of 2 mLL^{-1} water to break dormancy. Four seeds were planted for each pot and the seedlings were then thinned to two plants pot^{-1} at 4 days after emergence (DAE). Phosphorus fertilizer as triple superphosphate at the rate of 12.12 g P pot^{-1} and potassium fertilizer of muriate of potash (KCl) at 15.26 g K pot^{-1} were applied at 4 DAE. Gypsum ($CaSO_4$) at the rate of 9.58 g pot^{-1} was applied at 33 DAE. Pest and disease were controlled by weekly applications of carbosulfan [2-3-dihydro-2,2-dimethylbenzofuran-7-yl (dibutylaminothio) methylcarbamate 20%, w/v, water soluble concentrate] at 2.51 ha^{-1} , methomyl [S-methyl-N-((methylcarbamoyl)oxy) thioacetimidate 40% soluble powder] at 1.0 $kgha^{-1}$ and carboxin [5,6 dihydro-2-methyl-1.4oxath-ine-3carboxanilide 75% wettable powder] at 1.68 kg ha^{-1}.

The soil moisture for all pot was maintained at FC until 4 DAE for uniform germination. After 4 DAE, the irrigations treatments were initiated. Soil water level was maintained at FC until 60 DAE in well-watered treatment, and allowed to gradually reduce until reached predetermined level for the 1/3 AW (5.33%) stress treatment at 20 DAE. The water for additional applications was divided into four fractions, and the first fraction was given to the soil surface and the remaining fraction s through the plastic tubes into three holes levels. The soil moisture was maintained uniformly with no more than 1% moisture change of predetermined until 60 DAE.

In maintaining the soil moisture levels, water was added to the pots base on crop water requirement and surface evaporation which were calculated following the method describe by Doorenbos and Pruit (1992) and Singh and Russell (1981), respectively.

Calculation of total crop water use for each water treatment was calculated as the sum of transpiration and soil evaporation. Crop water requirement was calculated using the methods described by Doorenbos and Pruit (1992):

$$ETcrop = ETo \times Kc,$$

where ETcrop is a crop water requirement (mm/day), ETo is the evapotranspiration of the reference plant calculated by using class A pan evaporation method and Kc is a crop water requirement coefficient of peanut.

The surface evaporation was calculated by using the method from Singh and Rusell (1981):

$$Es = \beta \times (Eo / t),$$

where Es is the soil evaporation (mm), β is the light transmission coefficient, Eo is evaporation from class A pan (mm/day) and t is the day from last irrigation.

Weather data were obtained from the meteorological station closed to the experimental site and shown in figure 1.

Monitoring soil moisture content was observed at 40, 50 and 60 DAE by collected the soil samples from a depth between 0-60 cm using micro auger. Then placed the soil in an aluminum tins and sealed with paraffin to prevent moisture loss. Weight the samples and record as wet soil, the samples were then oven dried at 105°C for 48 h and recorded as dry soil. The soil moisture content was calculated by using the method from Black (1965):

soil moisture = [weight of wet soil – weight of dry soil / weight of dry soil]x100.

Soil moisture status showed reasonable management of soil moisture. A clear distinction among soil moisture levels was note at 40, 50 and 60DAE (figure 2).

Physiological data collection

Leaf was sampled from the nodal position two below the apex on the main stem during the morning period (0900-1100 h.), leaf samples were sealed within plastic bags, kept on ice and immediately transferred to the laboratory. The data were collected on 40, 50 and 60 DAE. Leaf samples were weight for fresh weight. Leaf areas were measured using an LI 3100 leaf area meter (LICOR, Lincon, USA). The leaf samples previously used for SLW measurements were placed in distilled water at 20 °C for 8h until saturated. The leaf samples were then dried at 80°C for 48h and dry weight was determined. SLW, the ratio of leaf dry weight to fresh leaf area (g/m^2) was calculated as

$$SLW = \text{Leaf dry weight (g)/Leaf area (m}^2\text{)}$$

The RWC was calculated as

$$RWC (\%) = [(FW-DW)/(TW-DW)] \times 100$$

Where FW is the sample fresh weight, TW is the sample turgid weight and DW is the sample dry weight.

Physiological data analysis

The data were subjected to analysis of variance and means were compared using least significant different (LSD). Also the SLW and RWC data were used to construct a dendrogram by using eucledian distance coefficient and clustering by unweighted paired group (UPGMA) method using NTSYSpc 2.01 software (Rohlf, 2000)

DNA isolation

Four peanut genotypes were grown in pots under greenhouse condition. Young leaves were collected at 9 DAE and placed in liquid nitrogen. Leaf tissues were ground to a fine powder and proceeded immediately to the DNA preparation by using the GenElute Plant Genomic DNA Miniprep kit (SIGMA- ALDRICH, USA). DNA was quantified on a 0.8 % agarose gel by visual comparison with lambda DNA standard (Invitrogen, USA) on ethidium bromide stained and subsequently diluted to 5 ng/ µl for PCR.

SSR analysis

Two hundred and sixty-five genomic SSR markers derived from different source were used to identify polymorphic among peanut genotypes (Table 1). PCR reaction for all primers were performed in 5µl. reaction volume in an ABI system 9700 thermal cycler (Applied Biosystem, USA) in 96-well PCR plates (Applied Biosystems, USA), consisting of 2 pmole of primer, 2 mM $MgCl_2$, 0.1mM dNTPs, 0.1 Unit of Taq DNA polymerase (Qiagen, Germany) and 1X PCR buffer (Qiagen, Germany). A touchdown PCR amplification with 3 min for initial denature cycle, followed by first five cycles of 94°C for 20 s, 60°C 20 s and 72°C for 30 with 1°C decreased in annealing temperature per cycle, then 30 cycle of 94°C for 20 s, constant annealing temperature at 55°C 30 s and 72°C for 30 s followed by final extension for 20 min at 72°C.

Electrophoresis and DNA banding analysis

SSR products were separated by electrophoresis on 6 % non-denaturing polyacrylamide gels (PAGE) at 650 volt for 2.5 – 3 hours in 1X TBE buffer on the Sequi-gen GT sequencing cell electrophoresis from Bio-RAD with the gel size 38 x 30 cm and 0.4 mm thickness, then SSR products were visualized through silver staining (figure 2). The size of fragments was estimated base on 100 bp DNA ladder from invitrogen (Invitrogen, USA). The presence or absence of amplicons in the genotype examined was scored as 1 or 0, respectively. A quality score was given to each primer by followed Ferguson et al. (2004) as 1 = unambiguous scoring; 2 = allele closed, but scoring possible; 3 = allele too close for accurate scoring when run on PAGE; 4 = weak amplification in at least one locus; 5 = variation due to absent of band; and 6 = variation in a fainter, secondary locus but of expected size range.

The polymorphic information content (PIC) of each microsatellite locus was determined as formular $PIC = 1- \Sigma P^2_i$ Where P_i is the frequency of the i_{th} allele in the genotypes examined (Weir, 1996). Allelic data obtained in 0 – 1 binary data for all alleles were used for computing the genetic similarity matrix by Dice's coefficient. The UPGMA method was used to construct a dendrogram by using NTSYSpc 2.01 software (Rohlf, 2000). Statistical stability of the branches in the cluster was performed by bootstrap analysis with 1,000 replicates using WINBOOT software (Yap and Nelson, 1996).

RESULTS AND DISCUSSION

Weather data

Weather data were obtained from a meteorological station adjacent to the experimental site. The experiment was conducted during the dry seasons from November 2006 to February 2007. There were maximum rainfalls of 1.0 mm at 17 DAE (Fig. 1). The seasonal means of maximum and minimum air temperatures ranged from 30.6 ^{o}C and 18.1^{o}C respectively. Daily pan evaporations ranged from 2.86 to 7.84 mm.

Physiological analysis

Peanut genotype did not showed statistical significant for SLW and RWC at 40 and 50 DAE (data not present). This may due to peanut is a drought tolerance crop in nature (Holbrook and Stalker, 2003), in case of peanut may need longer time for responses to drought. This similar to previous reported from Songsri et al. (2008) who found that the SLA under 1/3 AW and 2/3 AW were not significantly difference at 37 DAE because of early sampling date.

SLW was increased by drought stress but did not represent genetic significant between well watered and water limited conditions but peanut genotypes were significantly different in SLW in both water regimes at 60 DAE, ICGV 98353 showed the highest SLW (Figure 4), whereas KK 4 has the lowest SLW in drought conditions. The SLW, which related to leaf thickness, peanut genotypes with high SLW could maintain higher photosynthetic capacity. Peanut genotypes with high SLW also had higher chlorophyll contents and Rubisco enzyme. These enzymes are well known to involve in photosynthesis pathway (Arunyanark *et al.*, 2009 and Nautiyal *et al.*, 2002).

Craufurd et al. (1999) suggested that peanut genotype with low SLA could maintain higher WUE under water limited conditions. Under drought conditions SLA has negative correlation with WUE and HI. This trait also has high heritability and less effect of genotype x environment interaction (Songsri et al., 2008) thus, it is possible to use SLA to evaluate drought resistance in peanut.

RWC was significantly decreased under drought condition and also significantly among peanut genotype. ICGV 98324 could maintain highest RWC under water limited conditions, whereas KK 4 has the lowest RWC (Figure 5). This similar to previous report from Reddy et al. (2003) who found that RWC were ranged from 85- 90% in well-watering and it may drop to 30% under drought stress. Nautiyal et al. (1995) suggested that drought tolerance in peanut could be characterized by the maintenance of RWC under drought conditions. ICGV 98324 could maintain the highest RWC and it should be normal growth under drought stress.

The mean data from SLW and RWC were used to construct the dendrogram. The peanut genotypes were grouped into 2 clusters, which corresponded to the physiological traits. KK 4 (susceptible genotype) were grouped together with ICGV 98303 and ICGV 98324 was located in the same cluster as ICGV 98353 (Figure 6).

SSR analysis

SSR markers could be useful in discriminating between the narrow genetics based of cultivated peanut. Out of 265 genomic SSR markers, there were 182 markers that clearly appeared on PAGE and could be score (quality score as 1 and 2), 23 markers appeared as too close of alleles for accurate score, 29 markers have weak amplification, and 31 markers had variation due to absent of band (Table..). Only the markers appeared as clearly band (quality score as 1 and 2), were used to examined, the other were exclude from the analysis. Out of 182 clearly markers there were 89 markers could detect polymorphism among peanut genotypes (48.9%). The numbers of alleles of polymorphic markers were ranged from 1 – 6 with a mean of 2.7 alleles per locus. The PIC values were calculated separately from all individual marker loci and the range were varied from 038 – 0.78 with a mean of 0.5 (Table 2).

The data from 89 SSR polymorphic markers, then were scored as present (1) or absent (0) binary data. The genetics relationships among peanut genotypes were estimated using Dice coefficient. The bootstrap was performed with 1,000 replicated for supported the dendrogram and the value range from 61.3% to 98.9% (Figure 7). The dendrogram show that KK 4 (susceptible genotype) was clustered distinct from the others genotypes followed by ICGV 98353 while ICGV 98324 and ICGV 98303 were separated from the same node furthest from the KK 4. Due to the narrow genetics based in peanut, the low bootstrap may increase by added more of the polymorphic SSR marker (Barkley et al., 2007).

Conclusion

Drought condition could increase SLW and decrease RWC. ICGV 98353 had the highest SLW under well water and water limited conditions. ICGV 98324 could maintain the highest RWC under drought conditions. KK4 had the lowest SLW and RWC under water limited condition. Our result represented the SSR markers were effective for distinguished the genetics relationship between cultivated peanut. ICGV 98324 and ICGV 98303 were grouped in the same cluster furthest from the KK 4. Refer to both of physiological and markers data, KK 4 seem to be the most droughts sensitive genotype and it has genetics levels distinct from the others. The data from present study could be useful as a source of variation for construct the mapping population different in both of phenotypic and genotypic value for QTL analysis of physiological trait related to drought stress in breeding program.

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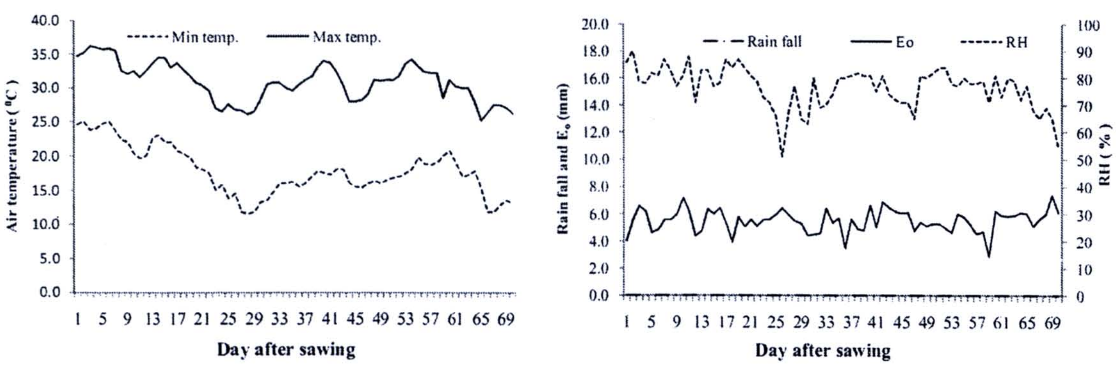

Figure 1 Maximum and minimum mean air temperature, total rainfall, evaporation (E_o) and relative humidity (RH) during growing season.

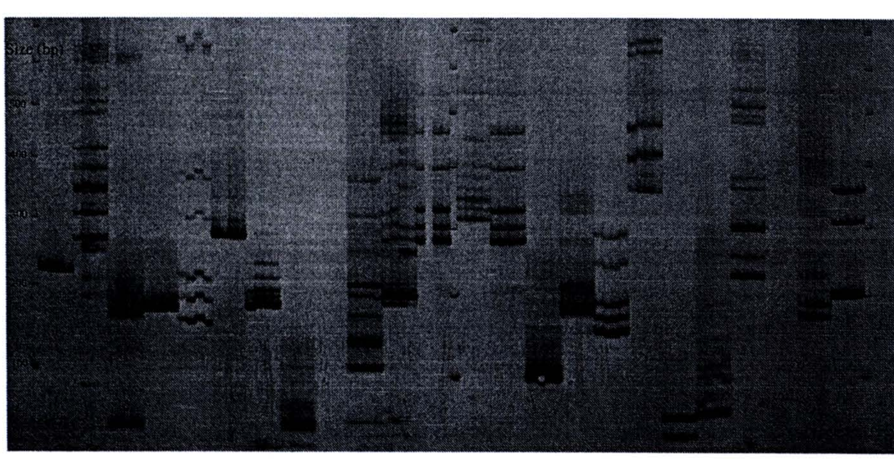

Figure 2 DNA profile obtained from SSR based of KK 4, ICGV 98324, ICGV 98303 and ICGV 98353 on 6% non-denature polyacrylamide gel stained with silver.

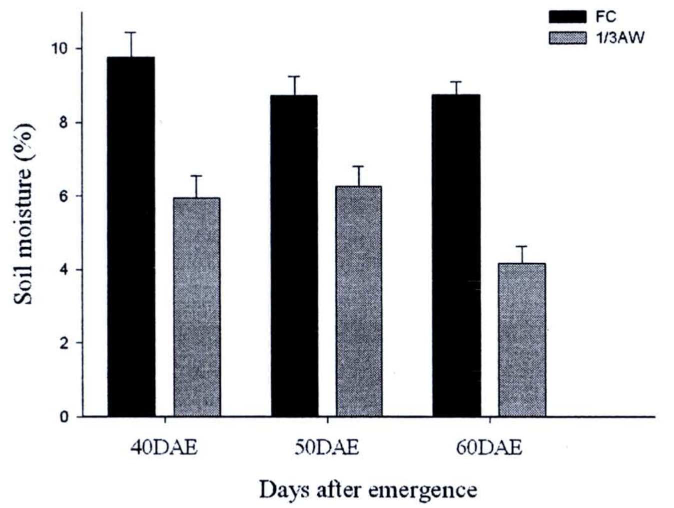

Figure 3 Soil moisture under field capacity (FC) and 1/3 available water (1/3 AW) at 40, 50 and 60 days after emergence.

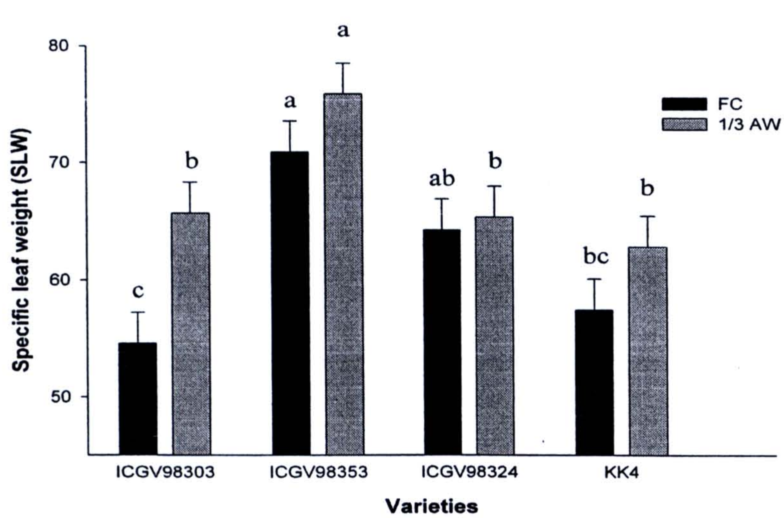

Figure 4: SLW of 4 peanut genotypes grown under different soil water regime (Field capacity; FC and 1/3 available water; 1/3AW) at 60 DAE.

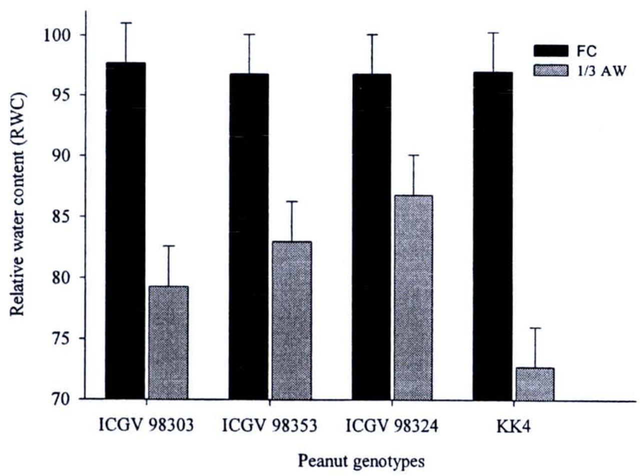

Figure 5: RWC of 4 peanut genotypes grown under different soil water regime (Field capacity; FC and 1/3 available water; 1/3AW) at 60 DAE.

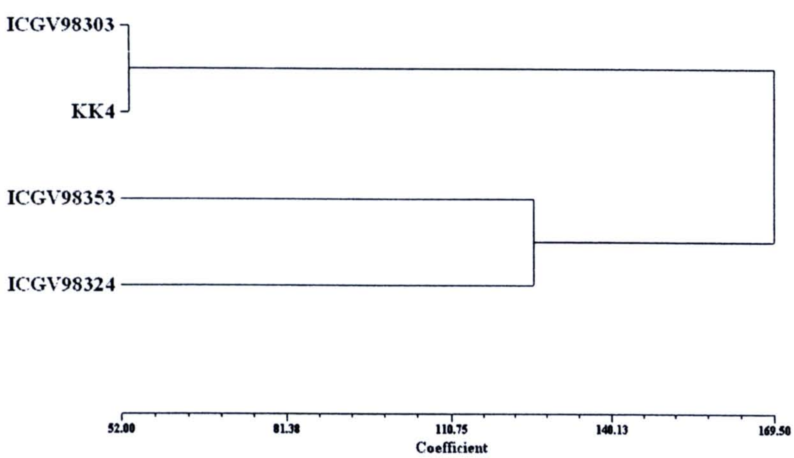

Figure 6: Dendrogram of the 4 peanut genotypes obtained from physiological data under drought stress condition.

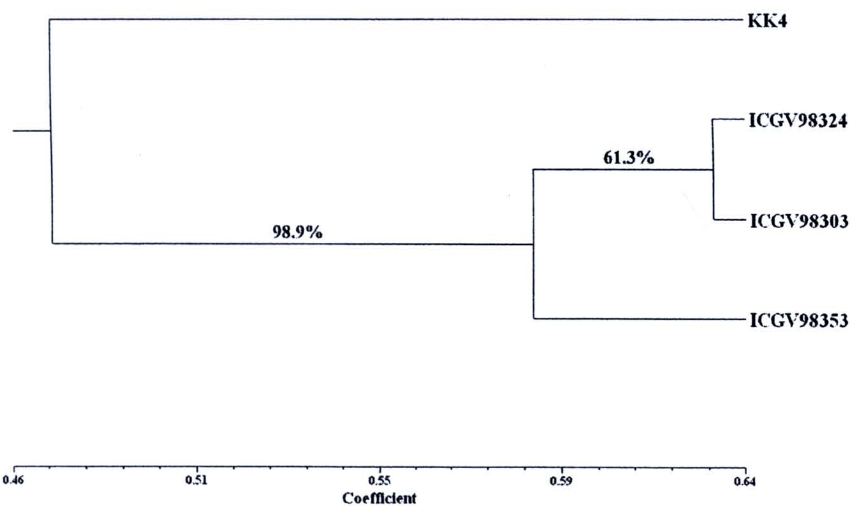

Figure 7: SSR based phylogenetic tree of the 4 peanut genotypes. The number on the branches represented the percentage of 1,000 bootstraping.

Table 1: List of SSR primers from different source were used to screen polymorphism among 4 peanut genotypes

Source	Primers name	number of primers
Cuc et al. (2008)	IPAHM	85
Moretzsohn et al. (2004, 2005)	Ah, gi, RN, ML, RI, TC, AC	143
He et al. (2003)	PM	15
Hopkins et al. (1999)	Ah	12
Gimenes et al. (2007)	Ah, Ag	10

Table 2: Number of alleles and polymorphic information content value (PIC) of the polymorphic gSSR primers among 4 peanut genotypes

No.	Primer name	No. of alleles	PIC	No.	Primer name	No. of alleles	PIC
1	Ah7	2	0.50	46	XIPAHM531	2	0.50
2	Ah11	4	0.72	47	XIPAHM659	3	0.59
3	Ah19	5	0.76	48	XIPAHM684	2	0.38
4	Ah30	2	0.38	49	XIPAHM023	2	0.50
5	Ah-075	2	0.50	50	XIPAHM82	2	0.38
6	Ah-097	2	0.38	51	XIPAHM93	3	0.63
7	Ah-193	4	0.72	52	XIPAHM108	3	0.63
8	Ah-229	3	0.63	53	XIPAHM123	2	0.38
9	Ah282	2	0.38	54	XIPAHM229	2	0.38
10	Ah-590	3	0.57	55	XIPAHM176	2	0.38
11	Ah-594	3	0.50	56	XIPAHM254	2	0.38
12	Ah-692	3	0.64	57	XIPAHM255	4	0.75
13	Ah-745	3	0.59	58	XIPAHM272	2	0.50
14	Ah4-24	2	0.38	59	XIPAHM287	2	0.38
15	Ah4-02	2	0.49	60	XIPAHM290	2	0.38
16	Ah4-11	2	0.50	61	XIPAHM302	2	0.38
17	Seq15D06	2	0.44	62	XIPAHM320	4	0.72
18	Seq16C07	3	0.63	63	XIPAHM333	2	0.50
19	Seq04E04	2	0.38	64	XIPAHM165	3	0.59
20	Seq04B11	3	0.61	65	XIPAHM171c	4	0.72
21	TC01E01	6	0.81	66	XIPAHM219	2	0.38
22	TC03G01	3	0.59	67	XIPAHM245	3	0.57
23	TC03G05	2	0.38	68	PM32	3	0.59
24	Ag140	2	0.50	69	PM35	2	0.38
25	TC11A02	2	0.38	70	PM36	4	0.66
26	TC07G10	2	0.38	71	PM42	2	0.49
27	TC09C06	3	0.59	72	PM45	2	0.50
28	TC09C08	2	0.38	73	PM69	2	0.38
29	TC09B08	3	0.63	74	TC00A01	4	0.75
30	TC11E04	2	0.38	75	TC01A08	6	0.76
31	AC1C11	2	0.38	76	TC01D12	3	0.59
32	AC2C12	2	0.38	77	TC01E05	2	0.50
33	AC1D11	2	0.50	78	TC02B09	2	0.38
34	AC1G11	6	0.78	79	TC02D08	3	0.63
35	AC2C08	4	0.66	80	TC02G05	2	0.38
36	AC02B03	4	0.67	81	TC03B04	3	0.59
37	XIPAHM552	2	0.38	82	TC04C11	4	0.70
38	XIPAHM354	2	0.38	83	TC04E09	2	0.50
39	XIPAHM395	2	0.38	84	TC04F02	2	0.38
40	XIPAHM407c	2	0.38	85	TC04F10	3	0.59
41	XIPAHM455	2	0.38	86	TC04G05	5	0.74
42	XIPAHM468	3	0.59	87	TC07D03	2	0.50
43	XIPAHM475	3	0.59	88	AC2C05	3	0.59
44	XIPAHM509	2	0.38	89	TC01B02	2	0.38
45	XIPAHM524	3	0.56				

SSR-based detection of genetic variability among peanut lines *(Arachis hypogaea* L.*)* different in Specific Leaf Weight and Relative Water Content

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Abstract

The objective of this study was undertaken to identify superior parental lines different in SLW and RWC and genetics level by using SSR markers. Four peanut genotypes and two water regimes (FC and 1/3 available water; 1/3 AW) were arranged in a split plot design with six replications. The data were recorded for specific leaf weight (SLW) and relative water content (RWC). Also four peanut genotypes were used to detected genetic diversity using 265 genomic SSR markers. Drought increased SLW and reduced RWC. ICGV 39353 had the highest SLW under well water and drought conditions. ICGV 98324 could maintain the highest RWC, whereas KK 4 seem to be the most sensitive to drought as it has the lowest SLW and RWC. SSR markers could effective to detect the narrow genetics based of cultivated peanut. There were 182 markers showed clearly appeared on PAGE. Out of 182 markers there were 89 markers could detect polymorphism among peanut genotypes (48.9%). The numbers of alleles were ranged from 1 – 6 with a mean of 2.7 alleles per locus. The polymorphic information content (PIC) values were varied from 038 – 0.78 with a mean of 0.5. The genetics relationship among peanut genotypes was estimated. KK 4 was clustered distinct from the others genotypes, whereas ICGV 98324 and ICGV 98303 were grouped in the same cluster furthest from the KK 4. The result from this study should be useful as a source of variation for development of mapping population for drought tolerance in peanut breeding program.

Key words: drought, water regime, polymorphism, genetic relationship

Introduction

Most peanut production areas are in the semi-arid tropics, which were affected by drought stress because unpredictable rainfall and rain distribution (Wright and Nageswara Rao, 1994). Drought stress can occurred at any time during crop growth, causing severe yield loss and poor seed quality. Therefore, attempts have been taken to improve peanut cultivars with tolerance to drought stress (Branch and Kvien, 1992). The conventional approach of breeding for drought tolerances has been based on pod yield. However, selection for pod yield is difficult because of large effect of genotype x environment interaction. The physiological traits associated with drought tolerance with high heritability and less affected of environmental variation have been suggested to determine the superior drought tolerant genotypes (Songsri et al., 2008). Specific leaf weight (SLW) and relative water content (RWC) are physiological parameters related to drought tolerance (Nigam et al., 2008; Nautiyal et al., 1995). Peanut genotypes with high SLW had higher Transpiration efficiency (Brown et al., 1996), this trait had low genotype x environment interaction and high heritability (Songsri et al., 2008). SLA (closely associated with SLW) also has negative relationship with stomatal conductance, Riburose – 1,5-bisphosphate Carboxylase - oxygenase (Rubisco) enzyme and leaves and carbon exchange rate (CEC). Under water limited conditions, peanut genotypes with low SLA could maintain higher RWC and normal growth (Nautiyal et al., 2002). Thus, it is possible to use SLA to evaluate drought tolerance in peanut (Vasanthi et al., 2006; Upadhyaya, 2005).

DNA markers have been applied in many plants breeding program. The progress of selection should be more rapid than the conventional methods because it can be conducted in early generation of segregating population with more accuracy (Stalker and Mozingo, 2001). The methods for identifying markers and traits associations are based on genotypic and phenotypic data from specific crossed population that shows a difference in the target traits and genetics level. The cultivated peanut has limited genetic variability. Based on many marker types studied such as isozyme, Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphic DNA (RFLP), Amplified Fragment Length Polymorphic (AFLP) could not detect the polymorphism or had very low polymorphic among cultivated peanuts because of narrow genetic base. (Halward et al., 1991; Halward et al., 1992; Singh et al, 1993; Lanham and Fenneil, 1992; Lack and Stalker, 1993; Gracia et al., 1995; Stalker et al., 1995 ; Kochert et al., 1996; He et al., 1997; He et al., 2001; Gimenes et al., 2002). Hopkin et al. (1999) first developed Simple Sequence Repeats (SSRs) technique for peanut, and this technique was further used successfully to detect polymorphism in cultivated peanuts (Krishna et al., 2004; Ferguson et al., 2004; Moretzsohn et al., 2004). These markers are small arrays of tandem arranged bases (one to six) spread throughout the genomes and are abundant, informative, and co-dominant in nature. Recently SSR markers have been recognized as useful tools in plant breeding program such as genetic diversity analysis (Cuc et al., 2008, He et al., 2005, Jiang et al., 2007 and Mace et al., 2006), germplasm management (Barkley et al., 2007), genome mapping, QTL analysis and applicable for marker assisted selection . (Chenault et al., 2009, Ferguson et al., 2004, Varshney et al., 2008 and Yan bin et al., 2008).

The present study was undertaken to identify superior parental lines different in SLW and RWC and genetics level by using SSR markers. The information should be useful as a source of variation for development of mapping population for drought tolerance in peanut breeding program.

MATERIALS AND METHOD

Plant materials

Four peanut genotypes consisting of three drought tolerance lines from ICRISAT (ICGV 98324, ICGV 98353 and ICGV 98303) and one genotype (KK 4) commonly grown in Thailand, a low SLW and RWC cultivar (Boontang et al., inpress; Akkasaeng et al., 2007) was used to determine the genotypic variability in SLW and RWC under well-water and water limited conditions.

Physiological measurement

The pot experiments were conducted under field conditions at the Field Crop Research Station of Khon Kaen University located in Khon Kaen province, Thailand (latitude 16° 28′ N, longitude 102° 48′ E, 200 m above mean sea level; AMSL) during November 2006 to April 2007. Using 2 x 4 factorial in randomized complete block design (RCBD) with 6 replications. Two soil moisture levels at field capacity (FC) (10.28%) and 1/3 available water (AW) (5.33%) were assigned as factor A and 4 peanut genotypes were assigned as factor B.

The soil on the experimental site pertains to the Yasothon series (loamy sand, Ocix Paleustults). The proportion of sand, silt and clay in the soil were 71.25%, 20% and 8.75%, respectively. A sandy loam soil with pH 5.58, 0.47 % organic matter and 0.0255% total nitrogen (N). Available phosphorus (P) was 7 ppm (Bray II & Molybdenum-blue method) and extractable potassium (K) and calcium (Ca) were 23.5 and 216.5 ppm respectively.

The plants were grown in column pot having a diameter 25 cm and height 70 cm. Each pot was filled to 10 cm from the top with 43.6 kg to created uniform bulk density. three holes were made at the height level 15, 30 and 45 cm from the bottom of each pot to facilitate watering and distribution of water. 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} seed before planting, and treated with ethrel 48% at the rate of 2 mLL^{-1} water to break dormancy. Four seeds were planted for each pot and the seedlings were then thinned to two plants pot^{-1} at 4 days after emergence (DAE). Phosphorus fertilizer as triple superphosphate at the rate of 12.12 g P pot^{-1} and potassium fertilizer of muriate of potash (KCl) at 15.26 g K pot^{-1} were applied at 4 DAE. Gypsum ($CaSO_4$) at the rate of 9.58 g pot^{-1} was applied at 33 DAE. Pest and disease were controlled by weekly applications of carbosulfan [2-3-dihydro-2,2-dimethylbenzofuran-7-yl (dibutylaminothio) methylcarbamate 20%, w/v, water soluble concentrate] at 2.51 ha^{-1} , methomyl [S-methyl-N-((methylcarbamoyl)oxy) thioacetimidate 40% soluble powder] at 1.0 $kgha^{-1}$ and carboxin [5,6 dihydro-2-methyl-1.4oxath-ine-3carboxanilide 75% wettable powder] at 1.68 kg ha^{-1}.

The soil moisture for all pot was maintained at FC until 4 DAE for uniform germination. After 4 DAE, the irrigations treatments were initiated. Soil water level was maintained at FC until 60 DAE in well-watered treatment, and allowed to gradually reduce until reached predetermined level for the 1/3 AW (5.33%) stress treatment at 20 DAE. The water for additional applications was divided into four fractions, and the first fraction was given to the soil surface and the remaining fraction s through the plastic tubes into three holes levels. The soil moisture was maintained uniformly with no more than 1% moisture change of predetermined until 60 DAE.

In maintaining the soil moisture levels, water was added to the pots base on crop water requirement and surface evaporation which were calculated following the method describe by Doorenbos and Pruit (1992) and Singh and Russell (1981), respectively.

Calculation of total crop water use for each water treatment was calculated as the sum of transpiration and soil evaporation. Crop water requirement was calculated using the methods described by Doorenbos and Pruit (1992):

$$ETcrop = ETo \times Kc,$$

where ETcrop is a crop water requirement (mm/day), ETo is the evapotranspiration of the reference plant calculated by using class A pan evaporation method and Kc is a crop water requirement coefficient of peanut.

The surface evaporation was calculated by using the method from Singh and Rusell (1981):

$Es = \beta \times (Eo / t)$,

where Es is the soil evaporation (mm), β is the light transmission coefficient, Eo is evaporation from class A pan (mm/day) and t is the day from last irrigation.

Weather data were obtained from the meteorological station closed to the experimental site and shown in figure 1.

Monitoring soil moisture content was observed at 40, 50 and 60 DAE by collected the soil samples from a depth between 0-60 cm using micro auger. Then placed the soil in an aluminum tins and sealed with paraffin to prevent moisture loss. Weight the samples and record as wet soil, the samples were then oven dried at 105°C for 48 h and recorded as dry soil. The soil moisture content was calculated by using the method from Black (1965):

soil moisture = [weight of wet soil – weight of dry soil / weight of dry soil]x100.

Soil moisture status showed reasonable management of soil moisture. A clear distinction among soil moisture levels was note at 40, 50 and 60DAE (figure 2).

Physiological data collection

Leaf was sampled from the nodal position two below the apex on the main stem during the morning period (0900-1100 h.), leaf samples were sealed within plastic bags, kept on ice and immediately transferred to the laboratory. The data were collected on 40, 50 and 60 DAE. Leaf samples were weight for fresh weight. Leaf areas were measured using an LI 3100 leaf area meter (LICOR, Lincon, USA). The leaf samples previously used for SLW measurements were placed in distilled water at 20 °C for 8h until saturated. The leaf samples were then dried at 80°C for 48h and dry weight was determined. SLW, the ratio of leaf dry weight to fresh leaf area (g/m^2) was calculated as

$$SLW = \text{Leaf dry weight (g)}/\text{Leaf area } (m^2)$$

The RWC was calculated as

$$RWC (\%) = [(FW-DW)/(TW-DW)] \times 100$$

Where FW is the sample fresh weight, TW is the sample turgid weight and DW is the sample dry weight.

Physiological data analysis

The data were subjected to analysis of variance and means were compared using least significant different (LSD). Also the SLW and RWC data were used to construct a dendrogram by using eucledian distance coefficient and clustering by unweighted paired group (UPGMA) method using NTSYSpc 2.01 software (Rohlf, 2000)

DNA isolation

Four peanut genotypes were grown in pots under greenhouse condition. Young leaves were collected at 9 DAE and placed in liquid nitrogen. Leaf tissues were ground to a fine powder and proceeded immediately to the DNA preparation by using the GenElute Plant Genomic DNA Miniprep kit (SIGMA- ALDRICH, USA). DNA was quantified on a 0.8 % agarose gel by visual comparison with lambda DNA standard (Invitrogen, USA) on ethidium bromide stained and subsequently diluted to 5 ng/ µl for PCR.

SSR analysis

Two hundred and sixty-five genomic SSR markers derived from different source were used to identify polymorphic among peanut genotypes (Table 1). PCR reaction for all primers were performed in 5µl. reaction volume in an ABI system 9700 thermal cycler (Applied Biosystem, USA) in 96-well PCR plates (Applied Biosystems, USA), consisting of 2 pmole of primer, 2 mM $MgCl_2$, 0.1mM dNTPs, 0.1 Unit of Taq DNA polymerase (Qiagen, Germany) and 1X PCR buffer (Qiagen, Germany). A touchdown PCR amplification with 3 min for initial denature cycle, followed by first five cycles of 94°C for 20 s, 60°C 20 s and 72°C for 30 with 1°C decreased in annealing temperature per cycle, then 30 cycle of 94°C for 20 s, constant annealing temperature at 55°C 30 s and 72°C for 30 s followed by final extension for 20 min at 72°C.

Electrophoresis and DNA banding analysis

SSR products were separated by electrophoresis on 6 % non-denaturing polyacrylamide gels (PAGE) at 650 volt for 2.5 – 3 hours in 1X TBE buffer on the Sequi-gen GT sequencing cell electrophoresis from Bio-RAD with the gel size 38 x 30 cm and 0.4 mm thickness, then SSR products were visualized through silver staining (figure 2). The size of fragments was estimated base on 100 bp DNA ladder from invitrogen (Invitrogen, USA). The presence or absence of amplicons in the genotype examined was scored as 1 or 0, respectively. A quality score was given to each primer by followed Ferguson et al. (2004) as 1 = unambiguous scoring; 2 = allele closed, but scoring possible; 3 = allele too close for accurate scoring when run on PAGE; 4 = weak amplification in at least one locus; 5 = variation due to absent of band; and 6 = variation in a fainter, secondary locus but of expected size range.

The polymorphic information content (PIC) of each microsatellite locus was determined as formular PIC = $1- \Sigma P^2_i$ Where P_i is the frequency of the i_{th} allele in the genotypes examined (Weir, 1996). Allelic data obtained in 0 – 1 binary data for all alleles were used for computing the genetic similarity matrix by Dice's coefficient. The UPGMA method was used to construct a dendrogram by using NTSYSpc 2.01 software (Rohlf, 2000). Statistical stability of the branches in the cluster was performed by bootstrap analysis with 1,000 replicates using WINBOOT software (Yap and Nelson, 1996).

RESULTS AND DISCUSSION

Weather data

Weather data were obtained from a meteorological station adjacent to the experimental site. The experiment was conducted during the dry seasons from November 2006 to February 2007. There were maximum rainfalls of 1.0 mm at 17 DAE (Fig. 1). The seasonal means of maximum and minimum air temperatures ranged from 30.6 °C and 18.1°C respectively. Daily pan evaporations ranged from 2.86 to 7.84 mm.

Physiological analysis

Peanut genotype did not showed statistical significant for SLW and RWC at 40 and 50 DAE (data not present). This may due to peanut is a drought tolerance crop in nature (Holbrook and Stalker, 2003), in case of peanut may need longer time for responses to drought. This similar to previous reported from Songsri et al. (2008) who found that the SLA under 1/3 AW and 2/3 AW were not significantly difference at 37 DAE because of early sampling date.

SLW was increased by drought stress but did not represent genetic significant between well watered and water limited conditions but peanut genotypes were significantly different in SLW in both water regimes at 60 DAE, ICGV 98353 showed the highest SLW (Figure 4), whereas KK 4 has the lowest SLW in drought conditions. The SLW, which related to leaf thickness, peanut genotypes with high SLW could maintain higher photosynthetic capacity. Peanut genotypes with high SLW also had higher chlorophyll contents and Rubisco enzyme. These enzymes are well known to involve in photosynthesis pathway (Arunyanark *et al.*, 2009 and Nautiyal *et al.*, 2002).

Craufurd et al. (1999) suggested that peanut genotype with low SLA could maintain higher WUE under water limited conditions. Under drought conditions SLA has negative correlation with WUE and HI. This trait also has high heritability and less effect of genotype x environment interaction (Songsri et al., 2008) thus, it is possible to use SLA to evaluate drought resistance in peanut.

RWC was significantly decreased under drought condition and also significantly among peanut genotype. ICGV 98324 could maintain highest RWC under water limited conditions, whereas KK 4 has the lowest RWC (Figure 5). This similar to previous report from Reddy et al. (2003) who found that RWC were ranged from 85- 90% in well-watering and it may drop to 30% under drought stress. Nautiyal et al. (1995) suggested that drought tolerance in peanut could be characterized by the maintenance of RWC under drought conditions. ICGV 98324 could maintain the highest RWC and it should be normal growth under drought stress.

The mean data from SLW and RWC were used to construct the dendrogram. The peanut genotypes were grouped into 2 clusters, which corresponded to the physiological traits. KK 4 (susceptible genotype) were grouped together with ICGV 98303 and ICGV 98324 was located in the same cluster as ICGV 98353 (Figure 6).

SSR analysis

SSR markers could be useful in discriminating between the narrow genetics based of cultivated peanut. Out of 265 genomic SSR markers, there were 182 markers that clearly appeared on PAGE and could be score (quality score as 1 and 2), 23 markers appeared as too close of alleles for accurate score, 29 markers have weak amplification, and 31 markers had variation due to absent of band (Table..). Only the markers appeared as clearly band (quality score as 1 and 2), were used to examined, the other were exclude from the analysis. Out of 182 clearly markers there were 89 markers could detect polymorphism among peanut genotypes (48.9%). The numbers of alleles of polymorphic markers were ranged from 1 – 6 with a mean of 2.7 alleles per locus. The PIC values were calculated separately from all individual marker loci and the range were varied from 038 – 0.78 with a mean of 0.5 (Table 2).

The data from 89 SSR polymorphic markers, then were scored as present (1) or absent (0) binary data. The genetics relationships among peanut genotypes were estimated using Dice coefficient. The bootstrap was performed with 1,000 replicated for supported the dendrogram and the value range from 61.3% to 98.9% (Figure 7). The dendrogram show that KK 4 (susceptible genotype) was clustered distinct from the others genotypes followed by ICGV 98353 while ICGV 98324 and ICGV 98303 were separated from the same node furthest from the KK 4. Due to the narrow genetics based in peanut, the low bootstrap may increase by added more of the polymorphic SSR marker (Barkley et al., 2007).

Conclusion

Drought condition could increase SLW and decrease RWC. ICGV 98353 had the highest SLW under well water and water limited conditions. ICGV 98324 could maintain the highest RWC under drought conditions. KK4 had the lowest SLW and RWC under water limited condition. Our result represented the SSR markers were effective for distinguished the genetics relationship between cultivated peanut. ICGV 98324 and ICGV 98303 were grouped in the same cluster furthest from the KK 4. Refer to both of physiological and markers data, KK 4 seem to be the most droughts sensitive genotype and it has genetics levels distinct from the others. The data from present study could be useful as a source of variation for construct the mapping population different in both of phenotypic and genotypic value for QTL analysis of physiological trait related to drought stress in breeding program.

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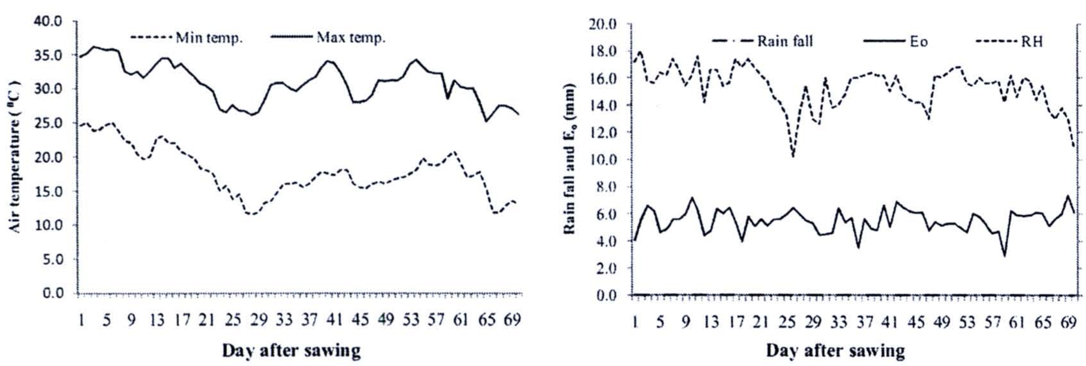

Figure 1 Maximum and minimum mean air temperature, total rainfall, evaporation (E_o) and relative humidity (RH) during growing season.

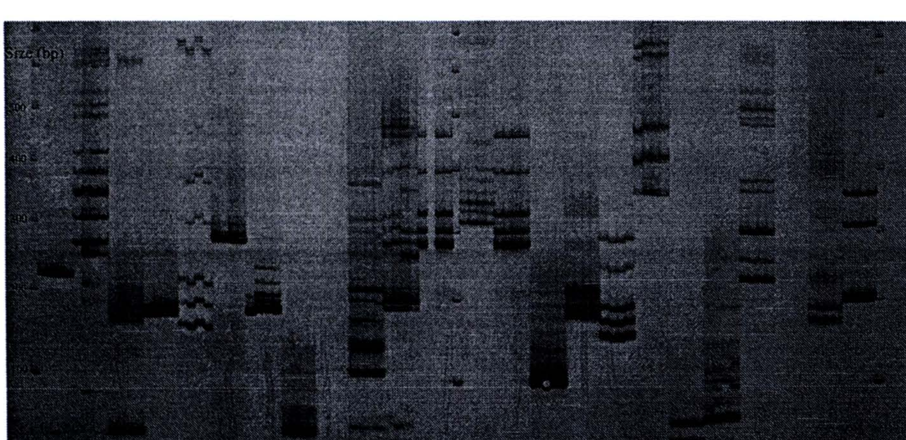

Figure 2 DNA profile obtained from SSR based of KK 4, ICGV 98324, ICGV 98303 and ICGV 98353 on 6% non-denature polyacrylamide gel stained with silver.

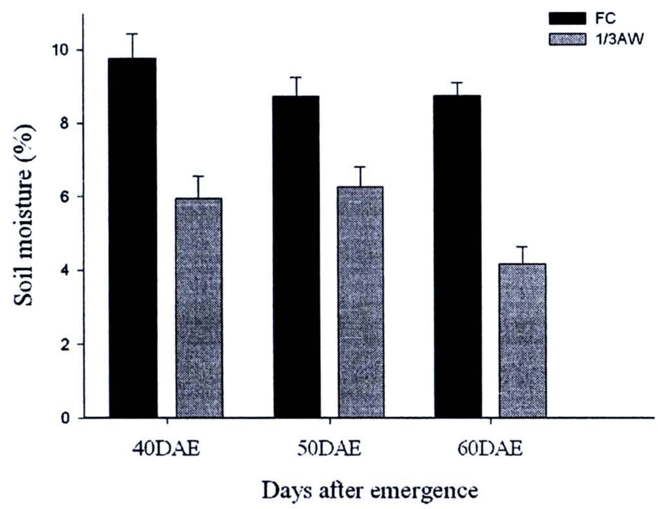

Figure 3 Soil moisture under field capacity (FC) and 1/3 available water (1/3 AW) at 40, 50 and 60 days after emergence.

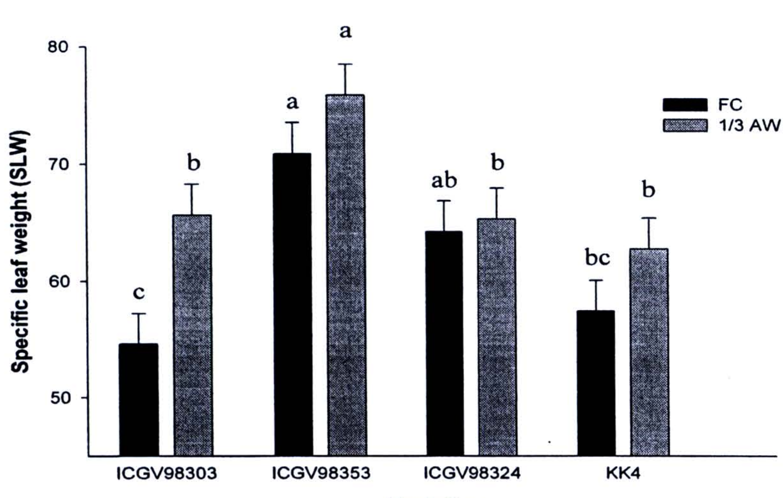

Figure 4: SLW of 4 peanut genotypes grown under different soil water regime (Field capacity; FC and 1/3 available water; 1/3AW) at 60 DAE.

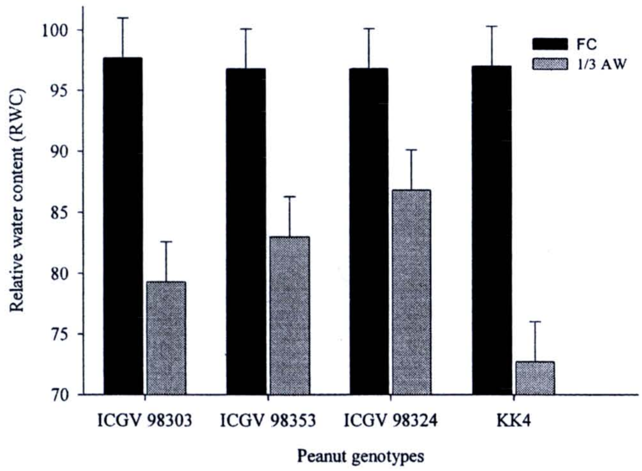

Figure 5: RWC of 4 peanut genotypes grown under different soil water regime (Field capacity; FC and 1/3 available water; 1/3AW) at 60 DAE.

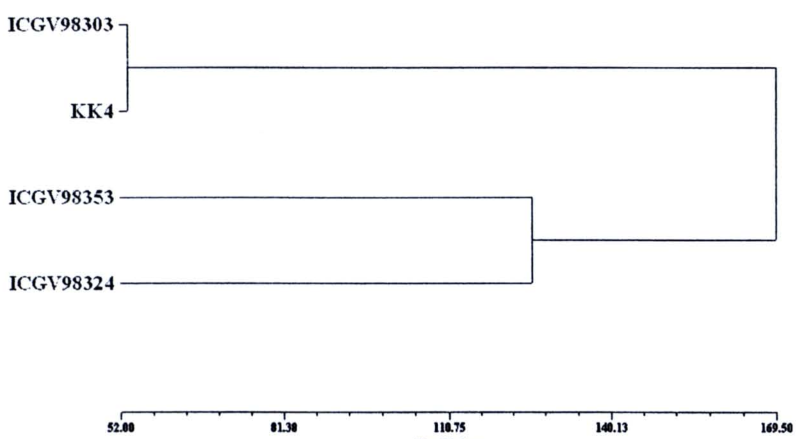

Figure 6: Dendrogram of the 4 peanut genotypes obtained from physiological data under drought stress condition.

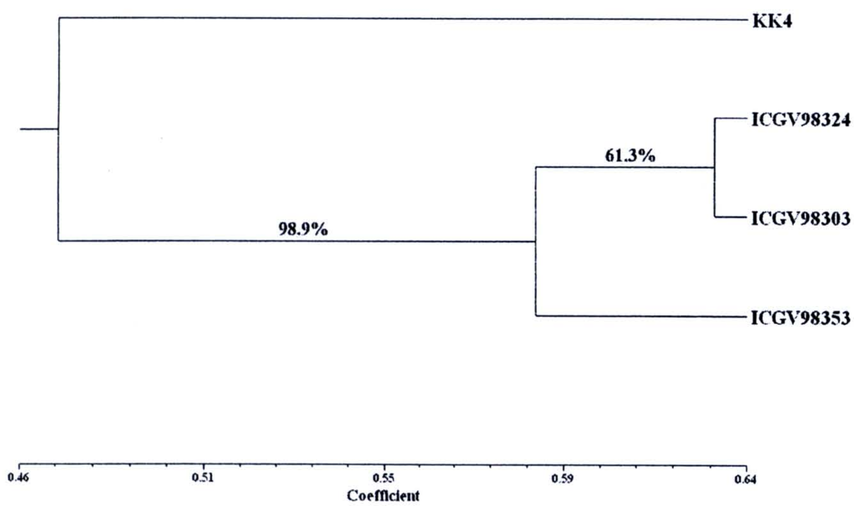

Figure 7: SSR based phylogenetic tree of the 4 peanut genotypes. The number on the branches represented the percentage of 1,000 bootstraping.

Table 1: List of SSR primers from different source were used to screen polymorphism among 4 peanut genotypes

Source	Primers name	number of primers
Cuc et al. (2008)	IPAHM	85
Moretzsohn et al. (2004, 2005)	Ah, gi, RN, ML, RI, TC, AC	143
He et al. (2003)	PM	15
Hopkins et al. (1999)	Ah	12
Gimenes et al. (2007)	Ah, Ag	10

Table 2: Number of alleles and polymorphic information content value (PIC) of the polymorphic gSSR primers among 4 peanut genotypes

No.	Primer name	No. of alleles	PIC	No.	Primer name	No. of alleles	PIC
1	Ah7	2	0.50	46	XIPAHM531	2	0.50
2	Ah11	4	0.72	47	XIPAHM659	3	0.59
3	Ah19	5	0.76	48	XIPAHM684	2	0.38
4	Ah30	2	0.38	49	XIPAHM023	2	0.50
5	Ah-075	2	0.50	50	XIPAHM82	2	0.38
6	Ah-097	2	0.38	51	XIPAHM93	3	0.63
7	Ah-193	4	0.72	52	XIPAHM108	3	0.63
8	Ah-229	3	0.63	53	XIPAHM123	2	0.38
9	Ah282	2	0.38	54	XIPAHM229	2	0.38
10	Ah-590	3	0.57	55	XIPAHM176	2	0.38
11	Ah-594	3	0.50	56	XIPAHM254	2	0.38
12	Ah-692	3	0.64	57	XIPAHM255	4	0.75
13	Ah-745	3	0.59	58	XIPAHM272	2	0.50
14	Ah4-24	2	0.38	59	XIPAHM287	2	0.38
15	Ah4-02	2	0.49	60	XIPAHM290	2	0.38
16	Ah4-11	2	0.50	61	XIPAHM302	2	0.38
17	Seq15D06	2	0.44	62	XIPAHM320	4	0.72
18	Seq16C07	3	0.63	63	XIPAHM333	2	0.50
19	Seq04E04	2	0.38	64	XIPAHM165	3	0.59
20	Seq04B11	3	0.61	65	XIPAHM171c	4	0.72
21	TC01E01	6	0.81	66	XIPAHM219	2	0.38
22	TC03G01	3	0.59	67	XIPAHM245	3	0.57
23	TC03G05	2	0.38	68	PM32	3	0.59
24	Ag140	2	0.50	69	PM35	2	0.38
25	TC11A02	2	0.38	70	PM36	4	0.66
26	TC07G10	2	0.38	71	PM42	2	0.49
27	TC09C06	3	0.59	72	PM45	2	0.50
28	TC09C08	2	0.38	73	PM69	2	0.38
29	TC09B08	3	0.63	74	TC00A01	4	0.75
30	TC11E04	2	0.38	75	TC01A08	6	0.76
31	AC1C11	2	0.38	76	TC01D12	3	0.59
32	AC2C12	2	0.38	77	TC01E05	2	0.50
33	AC1D11	2	0.50	78	TC02B09	2	0.38
34	AC1G11	6	0.78	79	TC02D08	3	0.63
35	AC2C08	4	0.66	80	TC02G05	2	0.38
36	AC02B03	4	0.67	81	TC03B04	3	0.59
37	XIPAHM552	2	0.38	82	TC04C11	4	0.70
38	XIPAHM354	2	0.38	83	TC04E09	2	0.50
39	XIPAHM395	2	0.38	84	TC04F02	2	0.38
40	XIPAHM407c	2	0.38	85	TC04F10	3	0.59
41	XIPAHM455	2	0.38	86	TC04G05	5	0.74
42	XIPAHM468	3	0.59	87	TC07D03	2	0.50
43	XIPAHM475	3	0.59	88	AC2C05	3	0.59
44	XIPAHM509	2	0.38	89	TC01B02	2	0.38
45	XIPAHM524	3	0.56				

Classification Root Distribution Patterns and Its Contributions to Yield in Peanut Genotypes under Mid-Season Drought Stress.

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Abstract

Peanut root distribution patterns are not well understood and have not been studied extensively. There is a lack of information on classification of root distribution patterns for many peanut genotypes and the relationship between rooting traits and yield under mid-season drought, which could be useful for peanut drought breeding programs. Forty peanut genotypes differencing drought tolerance level and source of origins were investigated under field conditions during dry season in 2007 and 2008 at Khon Kaen University, Thailand. A randomized complete block design with four replications was used in both years. Water regime was mid-season drought conditions as well-irrigated until withholding water from 50-83 days after planting (DAP) in the first season and 50-87 DAP in the repeated season. Top dry weight was observed at the most water stress date and harvest. Root was measured at the most water stress date same to top mass using auger method. The soil was sampled to a depth of 90 cm and was separated into three layers, including upper (0 to 30 cm), middle (30 to 60 cm) and deeper (60 to 90 cm) soil layers. For each peanut genotype the relative contribution to each layer was calculated and defined as root length density percentage (%RLD). Pod yield was observed at harvesting date and PHI was calculated as pod dry weight per biomass. The forty peanut genotypes were categorized as either high and low %RLD depending on the mean of %RLD in each layer for the three soil layers. These peanut genotypes were then categorized into six combinative groups, based on the high and low %RLD for each of the three layers. The relationship between %RLD in upper and middle soil layer were not affect yield under mid-season drought environment. Whereas the lower soil layer, the relationship between %RLD and yield traits was highly positive, indicating that %RLD in the

lower layer is an important trait that affects pod yield, PHI and top dry weight under mid-season drought conditions.

Keywords: root length density, root length density percentage, pod harvest index, water stress, top dry weight

1. Introduction

Peanut is largely grown under rain-fed conditions in the semi-arid tropics. In these conditions, drought is a major production constraint as rainfall is generally erratic and insufficient (Nageswara Rao et al. 1989, Reddy et al. 2003). Peanut productivity have different responses to water stress with different growth stage, water deficit during the seed filling phase as 50-80 days after planting (DAP) were a greatest reduction in yield but pod yield were increased by water deficit during the pre-flowering phase (Nageswara Rao et al., 1985; Nautiyal 1999; Meisner and Karnok 1992). The mechanisms of drought resistance in relation to above ground parts have been demonstrated in literature (Nageswara Rao et al., 1985; El Hafid et al., 1998; Nautiyal et al., 1999; Awal and Ikeda, 2002; Jongrungklang et al., 2008; Puangbut et al., 2009). But below ground part, the information on the responses of peanut has been slightly reported.

Drought resistance may be enhanced by improving the ability of the crop to extract water from the soil (Wright and Nageswara Rao 1994). Deep rooting, root length density (RLD) and root distribution have been identified as drought adaptive traits (Passioura 1983, Turner 1986, Dardanelli et al., 1997, Matsui and Singh 2003, Taiz and Zeiger 2006).

Under field conditions, peanut root is established both deeply and laterally in the soil profile early in the growth season, RLD significantly increased at each depth increment with DAP until 80 DAP under sufficient water conditions, the upper soil profile depth increment had the highest mean RLD (Ketring and Reid 1993). Whereas under drought conditions, root growth rate is significantly reduced in the upper soil layer during water stress from 20 to 50 DAP compared sufficient irrigation (Meisner and Karnok 1992)

The peanut genotypes having the higher RLD at lower soil depth enhanced drought tolerance and such response can help peanut genotypes to obtain high pod yield and harvest index, indicating that the genotypes which were classified as drought responsive as they increased RLD in deeper soil layer in response to long season drought environment (Songsri et al., 2008). Pandey et al. (1984) reported that drought increased RLD in the lower soil profile of a peanut genotype (Kidang). In contrast, Robertson et al. (1980) showed that RLD of a peanut (Florunner) was not affected by differential water managements.

Peanut root distribution patterns are not well understood and have not been studied extensively. The results reported so far have been limited to the experiments under chamber conditions, and more previous studies investigated in a few peanut genotypes. Especially, there is a lack of information on classification of root distribution patterns for many peanut genotypes under mid-season drought, which could be useful for peanut drought breeding programs. Thus, the aims of this study were to classify the root distribution pattern of peanut genotypes under mid-season drought, and to determine of the relationships between RLD in different soil depths and yield under these conditions.

2. Materials and Methods

2.1. Experimental design and treatments

The experiment was conducted under field conditions at Field Crop Research station of Khon Kaen University located in Khon Kaen province, Thailand (latitude 16° 28′ N, longitude 102° 48′ E, 200 m. above sea level) during December 2007 to May 2008 and repeated during November 2008 to April 2009. The soil type is Yasothon series (Yt: fine-loamy; siliceous, isohypothermic, Oxic Paleustults). A randomized complete block design with four replications was used in both years. Plot size was 3 x 5 m with spacing of 50 cm between rows and 20 cm between plants.

The treatments of this study were forty peanut cultivars which differencing drought tolerance level and source of origins. Nine genotypes which having different drought tolerance level were identified using the reduction percentage of total dry matter as reported by Jongrungklang et al. (2008) (Table 1; no entries 1-9), received from the United State Department of Agriculture (USDA). Eleven commercially released cultivars in Thailand (KKU 40, KKU 60, KKU 1, KKU 72-1, KK 6, KK 4, KK5, KS 2, KK 60-2, KK 60-3, Tainan 9), these peanut cultivars were also investigated in this study (Table 1; no entries 10-20). KK 60-3 is a Virginia-type peanut cultivar sensitive to drought for pod yield, while Tainan 9 is a Spanish-type peanut cultivar having low dry matter production under drought conditions (Vorasoot et al., 2003). Eight elite drought resistant lines (ICGV 98300, ICGV 98303, ICGV 98305, ICGV 98308, ICGV 98324, ICGV 98330, ICGV 98348 and ICGV 98353) kindly provided by International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) in India (Table 1; no entries 21-28), the drought resistant lines from ICRISAT had been selected because of high total dry matter and pod yield under drought stress experiments (Nageswara Rao et al., 1992; Nigam et al., 2003; Nigam et al., 2005). One (Tifton-8) is a Virginia-type drought-resistant line (Coffelt et al. 1985) introduced from USDA (Table 1; no entry 29). Whereas, Eleven cultivars had been selected because having different dry matter production, harvest index and specific leaf area under well-water conditions (data from our previous study), the hybrid lines in Table 1 number entries 30-37 provided from USDA, and the last three cultivars (Table 1; no entries 38-40) received from China.

2.2. Crop management

Sub-soiling was done to destroy the hard pan soil from 0-60 cm of soil depth and disc plowing was performed three times to prepare soil suitable for the experiment. Lime at the rate of 625 kg ha^{-1} was incorporated into the soil during soil preparation to adjust soil pH. Seeds were treated with captan (3a,4,7,7a-tetrahydro-2-[(trichloromethyl)thio]-1H-isoindole-1,3(2H)-dione) at the rate of 5 gm kg^{-1} seed before planting, and the large seed lines were treated with ethrel 48% at the rate of 2 ml l^{-1} water to break seed dormancy before planting. Three seeds were planted per hill and the seedlings were thinned to one plant per hill at 15 days after planting (DAP). Nitrogen fertilizer as urea was applied at a rate of 23.4 kg ha^{-1}, phosphorus fertilizer as triple superphosphate at 24.7 kg P ha^{-1} and potassium fertilizer as muriate of potash (KCl) at 31.1 kg K ha^{-1} were also applied at 15 DAP. Gypsum ($CaSO_4$) at the rate of 312 kg ha^{-1} was applied at the 45 DAP to improve pod development.

Weeds were controlled by an 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. Carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-ylmethylcarbamate 3% granular) was applied at the pod setting stage to control soil insects. 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 2.5 l ha^{-1}, methomyl [*S*-methyl-*N*-((methylcarbamoyl)oxy) thioacetimidate 40% soluble powder] at 1.0 kg ha^{-1} and carboxin [5,6-dihydro-2-methyl-1,4-oxathine-3-carboxanilide 75% wettable powder] at 1.68 kg ha^{-1}.

Before planting, sprinkler irrigation system was installed to supply water. Water regime in this experiment was mimic mid-season drought in natural field. Peanut might represent maximum drought tolerance potential under mid-season drought conditions, drought during pod filling and seed filling development affect significantly reducing pod yield (Nageswara Rao et al., 1985, Meisner and Karnok, 1992). Therefore, all plots were supplied with water to obtain field capacity moisture level of the depth of 60 cm from planting to 50 DAP. After 50 DAP, water was withheld until 83 DAP in first season. For repeated season, irrigation was withheld from 50 to 87 DAP due to this season needs to mimic growth stage during stop irrigation of all peanut genotypes as the first season. Therefore, thermal degree day accumulation was calculated in this experiment for predicted crop growth stage. After drought period time, all plots were re-watering at F.C. moisture level and control this level until harvest.

2.3. Soil moisture content and meteorological conditions

The soil water status was monitored at 7 day intervals using a neutron moisture meter (Type I.H. II SER. N^{o}NO 152, Ambe Diccot Instruments CO.Ltd.,England). An aluminum access tube was installed between rows in each plot sixteen-second neutron moisture meter readings were made at depth 30, 60 and 90 cm (30cm intervals). Rainfall, relative humidity (RH), evaporation (E_0), maximum and minimum temperature and solar radiation were recorded daily from sowing until harvest by a weather station located 100 m away from the experimental field.

2.4. Top dry matter

Shoot dry weight and leave dry weight were observed at the most water stress date, the first season as 83 DAP, and 87 DAP for the second season. Five plants were collected for each plot and observed fresh weight. The samples were separated shoot and leave and observed fresh weight. After that sample were conducted to oven dry (temperature 80 C^{o} 48 hours or until constant weight) and were collected dry weight data. At harvest, ten plants were explored to the sun and sub-sampling. Then the samples were conducted to oven dry and determined dry weight.

2.5. Root length density percentage

Root sample was observed at the most water stress date same to top mass using auger method. Each plot was collected root length density as two positions, at the center of plants in the row and between row positions. Root samples were taken to 90 cm depth and separated into six layers as 0-15 cm, 15-30cm, 30-45 cm, 45-60cm, 60-75cm and 75-90 cm. Root samples of each layer were washed manually with tap water to remove soil from root sample. Root length was analyzed by Winrhizo program (Winrhizo Pro (s) V. 2004a by Regent Instruments INC).

Root length density (RLD) was calculated as the ratio between root length (cm) and soil volume (cm3). Each peanut genotype, the relative contribution to each layer was calculated and defined as root length density percentage (%RLD). % RLD from the first (0–15 cm) and second (15–30 cm) layer were added together and defined as a single 0 to 30 cm layer (upper soil layer), third (30–45 cm) and forth (45–

60 cm) layers were defined as a single 30 to 60 cm layer (middle soil layer), while %RLD at the deeper layers (fifth and sixth layers) were combined to form a single 60 to 90 cm layer. %RLD was separated into three layers, including upper, middle and deeper layers base on the layer of soil moisture detection by neutron moisture meter readings.

2.6. Pod yield and pod harvest index (PHI)

For each plot, plants in area of 9.0 m^2 were harvested at maturity (R8) (Boote 1982), pod yield were weighted after air drying to approximately 8% moisture content then calculated pod dry weight per harvest area. PHI was calculated as pod dry weight per biomass.

2.7. Statistical analysis

Data for each year were analyzed separately because the G x E interaction was apparently significant (data not shown), indicating that the response of peanut genotypes was different between two seasons. Calculation procedures were done using MSTAT-C package (Bricker, 1989). The data were subjected to analysis of variance according to randomize complete block design. Mean comparison was done based on Dancan's Multiple Range Test (DMRT) (Gomez and Gomez 1984).

Simple correlation was used to determine the relationship between pod yield and top dry weight at the most stressed date, top dry weight at harvest and pod harvest index and, the relationship between root length density percentage in each layer and pod yield, top dry weight at the most stressed date, top dry weight at harvest and PHI.

In each year, %RLD were categorized as either high and low using the mean of %RLD in each layer of all Forty peanut genotypes. And then these peanut genotypes were defined as combinative groups based on the high and low %RLD for each of the three soil layers as upper, middle and deeper layer

3. Results and Discussion

3.1. Soil moisture content and meteorological conditions

Soil moisture contents both two seasons were significantly reduced during withhold irrigation at the soil depth of 30 cm (Figure 1) compare with soil moisture content before stop irrigation. The reducing was smaller at 60 cm, and the smallest reduction at 90 cm. The results showed adequate control of water treatments as mid-season drought conditions.

The first experiment was conducted during December 2007- May 2008. Mean air temperatures ranged from 32.1 to 21.0°C during crop season, and there was no rainfall during drought stress period and all rainfall in this season as 459.2 mm. Whereas repeated season was conducted during November 2008- April 2009, there were mean air temperatures ranged from 31.6 to 19.8°C during crop season, and there was no rainfall during drought stress period and all rainfall in this season as 60.5 mm (Figure 2). Even though rainfall was large amount, but it was not occurred during mid-season drought period. Therefore, rainfall would not have effect on this investigation.

3.2. Root distribution patterns of peanut

Forty peanut genotypes were categorized as either high and low %RLD depending on the mean of %RLD of each layer for the three soil layers as upper (0-30 cm soil depth), middle (30-60 cm soil depth) and lower (60-90 cm soil depth). For the first season, the range for the high %RLD genotypes for the upper layer was 67.3-56.1%, whereas the range for the low %RLD genotypes was 54.9-39.1%. For the middle layer, the range of the high %RLD genotypes was 33.4-27.2%, while the range for the low %RLD was 27.0-17.8%. For the lower layer, the range for the high %RLD genotypes was 28.7-17.4%, while the range for the low %RLD genotypes was 17.0-5.6% (Table 2). For the second season, the range for the high %RLD genotypes for the upper layer was 77.8-50.5%, whereas the range for the low %RLD genotypes was 49.8-33.5%. The range of the high %RLD genotypes represented as 41.4-31.1% in middle layer, whereas the range of the low %RLD genotypes was 30.7-15.1%. In lower layer, there was 32.0-19.5% for the range of the high %RLD genotypes, while the range of the low %RLD genotypes was defined as 19.0-6.5% (Table 2).

RLD and %RLD were the highest in the top soil, and they gradually reduced with increasing soil depths in sufficient water conditions, (Pandey et al., 1984; Ketring and Reid 1993) and long-term drought conditions (Songsri et al., 2008). In this study, %RLD under mid-season drought was reduced with increasing soil depths same to other conditions. These observed results was rather high variation, this may have been due to a great number of genotypes. The impact of varying in peanut genetics for root growth was showed in Songsri et al. (2008), who investigation in changing %RLD at the 40 to 100 cm soil layer under long period drought conditions reported that eleven peanut genotypes were different %RLD in deeper soil layer in three conditions as adequate water, mild water stress and severe water stress. Furthermore, Benjamin and Nielson (2006) demonstrated that water stress affect in smaller proportion of chickpea and field pea root in upper soil layer (0.23 m) than adequate water conditions, and suggested that these species is suited for dryland crop.

Therefore, it might have relationship between %RLD and yield under drought environments.

Forty peanut genotypes were categorized into six combinative groups (Table 3), based on the high and low %RLD for each of the three layers as upper, middle and deeper layer. Five peanut genotypes were defined as having high %RLD in upper and middle layers but low %RLD in lower layer (HHL)(Figure 3a), Tainan 9, KK60-2, KS2, KK 4 and 35 Grif 13932 were classified in this group (Table 3). Four genotypes categorized as having high %RLD at upper but low %RLD at middle and lower layers (HLL) (Figure 3b), 306 PI 430237, 248 Grif 13911, 97 PI 158854 and 303 PI 430230 were peanut lines which classified as HLL group (Table 3). Five peanut lines were classified as low %RLD in upper and lower layers but high %RLD in middle layer (LHL) (Figure 3c), peanut genotypes were determined as LHL group were 187 PI 433352, 283 PI 234375, KKU 40, ICGV 98305 and 100 PI 162604 (Table 3). Ten peanut genotypes were defined as low %RLD in upper layer but high %RLD in middle and lower layers (LHH) (Figure 3d), there were 101 PI 268659, 89 PI 157549, KK6, KK60-3, KKU 60, ICGV 98300, ICGV 98330, 102 PI 268660, Taiwan 2 and Luhua 11 (Table 3). Seven peanut genotypes were defined as low %RLD in upper and middle layers but high %RLD in deepest layer (LLH) (Figure 3e). 269 PI 157542, KKU 72-1, ICGV 98324, ICGV 98348, 106 PI 268949, Taiwan 1 and Tifton–8 were peanut lines which classified as LLH group (Table 3). Two peanut lines which classified as high %RLD in upper and lower layers but low %RLD in middle layer (HLH) (Figure 3f), ICGV 98353 and 204 PI 442572 were defined as HLH group (Table 3).

In these results, under middle crop drought conditions, nineteen peanut genotypes have a great %RLD in lower soil layer as LHH, HLH and LLH. These observations agreed with Pandey et al. (1984), who reported that drought environment increased RLD of peanut in the lower soil profile. However, this previous report observed only a peanut genotype. Whereas, the peanut genotypes group which having a small %RLD in deeper soil layer under these conditions as HHL, LHL and HLL were fourteen genotypes. Even though this investigation has different drought conditions with Songsri et al. (2008), but these results conformed to this previous report that different peanut genotypes responded differently for RLD in deeper soil when subject in long period environment.

However, this observation, there were seven peanut lines as 12 PI 430233, 5 PI 313160, KK 5, KKU 1, ICGV 98303, ICGV 98308 and 3 PI 313157 that could not classified root distribution patterns, due to those genotypes were no consistent %RLD pattern in each layer between both seasons. Excepting the effect of varying genetics, field cultivation along with environment conditions apparently affected root growth such as soil preparing and strength (Ketring and Reid 1993; Robertson et al., 1980).

3.3. Pod yield, top dry weight and PHI under mid-season drought conditions and relationship of those traits

In this study, pod yield, top dry weight and pod harvest index (PHI) were significance between two seasons (data not shown). Therefore data were showed as separating year (Table 4 and 5).

All traits, top dry weight at the most drought stressed date (the first season at 83 DAS and the second season at 87 DAS), top dry weight at harvest, pod yield and PHI showed highly difference in among peanut lines for both seasons under mid-season stressed (Table 4 and 5).

According to the results, all traits contributed a poor productivity under mid-season drought conditions. Pod yield of peanut is extremely decreased by severe drought such mid-season (Pallas et al., 1979; Nageswara Rao et al., 1985; Meisner and Karnok 1992; Nautiyal 1999). However, this experiment could present some lines which produced pod yield, top weight and PHI as fairly good in these conditions. The top ten lines which showing high pod yield under mid-season drought conditions and still consistency for both seasons were KKU 60, Luhua 11, Taiwan 1, KKU 72-1, Tifton – 8, 106 PI 268949, 101 PI 268659, ICGV 98353, ICGV 98305 and KK 6. For top dry weight at the most drought stressed date, the top seven lines which having high top weight under these conditions and consistent values for both seasons were KKU 60, KKU 40, Luhua 11, Taiwan 1, KKU 72-1, 101 PI 268659 and ICGV 98300. Whereas top dry weight at harvest , the top five lines which presenting a high value in severe drought such mid-season water stress and consistent values for both seasons were KKU 60, KKU 72-1, Tifton-8, 101 PI 268659 and ICGV 98353. PHI, there were seven lines showing consistent high PHI for both seasons as KKU 60, Luhua 11, Taiwan 1, KKU 72-1, 106 PI 268949, ICGV 98348 and 269 PI 157542.

The following the top range of genotypes that still appeared fairly good for pod yield, top dry weight and PHI under mid-season drought conditions. There were seven peanut genotypes which were defined as drought tolerance genotypes by previous report (Coffelt et al., 1985, Nageswara Rao et al., 1992, Nigam et al., 2003, Nigam et al., 2005, Jongrungklang et al., 2008). Five genotypes had never been estimated before. And two genotypes were classified as moderate drought tolerance genotypes (Jongrungklang et al., 2008). Certainly, there were no susceptible genotypes for the top range of genotypes as having a good maintainable yield under these conditions.

The relationship of those traits under mid season drought conditions were defined in this study. Pod yield had highly correlation coefficients with top dry weight and PHI, the correlation between pod yield and top dry weight at the most stressed date were 0.49 and 0.51 in the first and the second seasons respectively (Table 6) whereas top dry weight at harvest and pod yield had highly correlations as 0.89 (the first season) and 0.58 (the second season) (Table 6). Even these observations was conducted under mid-season drought conditions, but the relationship between pod yield and top dry weight supported with previous report which subjected in different conditions as reporting in Del Rosario and Fajardo (1988). For PHI, the correlation between pod yield and PHI were highly positive relation as 0.94 and 0.83 in the first and the second season, respectively (Table 6). Probably, PHI is the most important trait that contributed to pod yield productivity under mid-season drought. Harvest index determined pod yield under sufficient water conditions (Duncan et al., 1978) and moisture-limited environment (Passioura 1977; Nautiyal et al., 2002).

3.4. Relationship between %RLD and pod yield, top dry weight and PHI

Simple correlation coefficients between %RLD for three soil depths and pod yield, top dry weight and PHI were calculated in each season.

In upper layer (0-30 cm), the relationships between %RLD and pod yield were negative for both seasons (Figure 4 a, d). However, the first season, there was not significant correlation. This indicated %RLD in upper soil layer could not affect pod yield under severe drought stress such mid-season drought environment. As middle layer (30-60 cm), in these conditions, there were no significant correlations for both seasons (Figure 4 b, e), indicating that %RLD in middle soil depth layer did not affect pod dry weight at harvest. Whereas lower layer (60-90 cm), %RLD were positively correlation with pod yield for both seasons (r = 0.42 and 0.58 in the first and the second seasons, respectively) (Figure 4 c, f), meaning that the amount of RLD at lower layer is one important trait to distribute pod yield under mid-season drought conditions.

The relationships between %RLD and top weight at the most drought stressed date, top dry weight at harvest and PHI, all traits had a similar response under these water stress conditions. That was negative correlation between %RLD and those traits at upper layer excepting PHI in the first season (Table 7).Whereas, middle layer, there were no correlation for all variables. But, both seasons, %RLD was positive correlation with top weight at the most drought stressed date, top dry weight at harvest and PHI in lower layer.

According to the relationship between %RLD of deeper soil layers and pod yield, top dry weight and PHI, indicating that the genotypes partitioning root length density to deep soil could give higher pod yield, top dry weight and PHI under mid-season drought conditions than the genotypes which did not. The relationships between %RLD in deeper soil as 60-90 cm and yield traits under mid-season drought have not reported previously. However, these results is along with Songsri et al. (2008), who reported that drought avoidance by change root distribution into deep soil is one of the mechanisms that helps peanut to maintain pod yield and HI under long period drought environment.

4. Conclusions

In summary, peanut genotypes were categorized into six combinative groups, based on the high and low %RLD for each of the three layers as upper, middle and deeper layer. The six combination groups were HHL, HLH, HLL, LHH, LHL and LLH. Forty peanut genotypes have different top dry weight, pod yield and PHI under mid-season stressed. The relationship between %RLD in upper and middle soil layer were not affect yield under mid-season drought environment. Whereas the lower soil layer, the relationship between %RLD and yield traits was highly positive, indicating that %RLD in the lower layer is an important trait that affects pod yield, PHI and top dry weight under mid-season drought conditions. Moreover, these observations also found that PHI might be the most important trait that distributed to pod yield productivity under mid-season drought.

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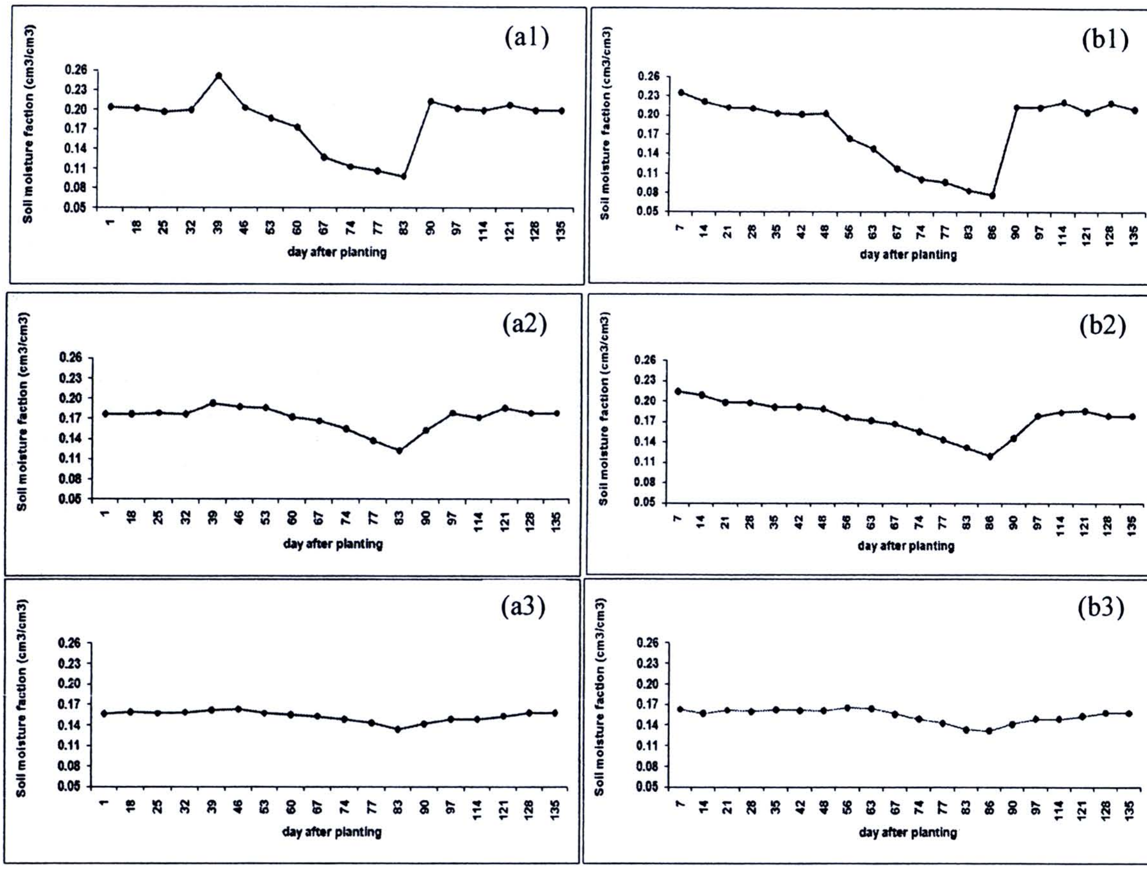

Figure 1 Volumetric soil moisture (fraction) in mid-season drought the experiment were conducted at Khon Kaen during December 2007- May 2008 1[st] season at 30cm (a1), 60cm (a2) and 90 cm (a3) and repeat during November 2008- April 2009 2[nd] season at 30cm (b1), 60cm (b2) and 90 cm (b3).

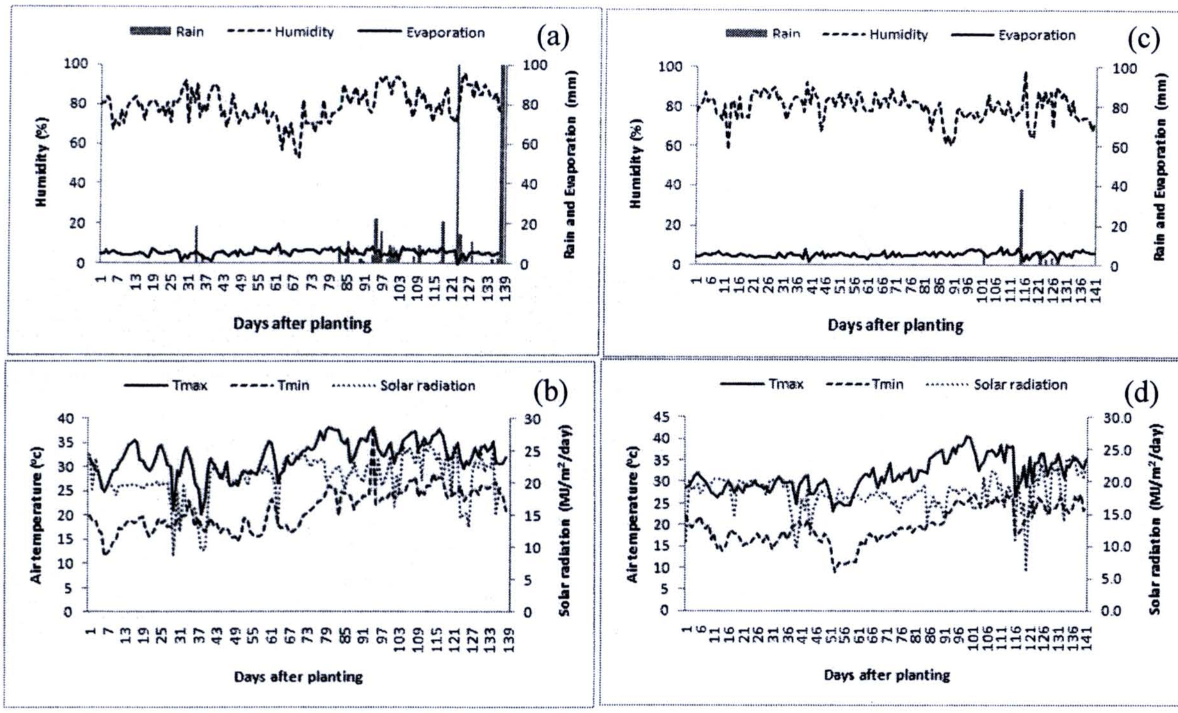

Figure 2 Rain fall, humidity (RH), evaporation (E0), maximum (Tmax) and minimum (Tmin) temperature and solar radiation during December 2007- May 2008 (a,b) and during November 2008- April 2009 (c,d) at the meteorological station, Khon Kaen University, Khon Kaen, Thailand.

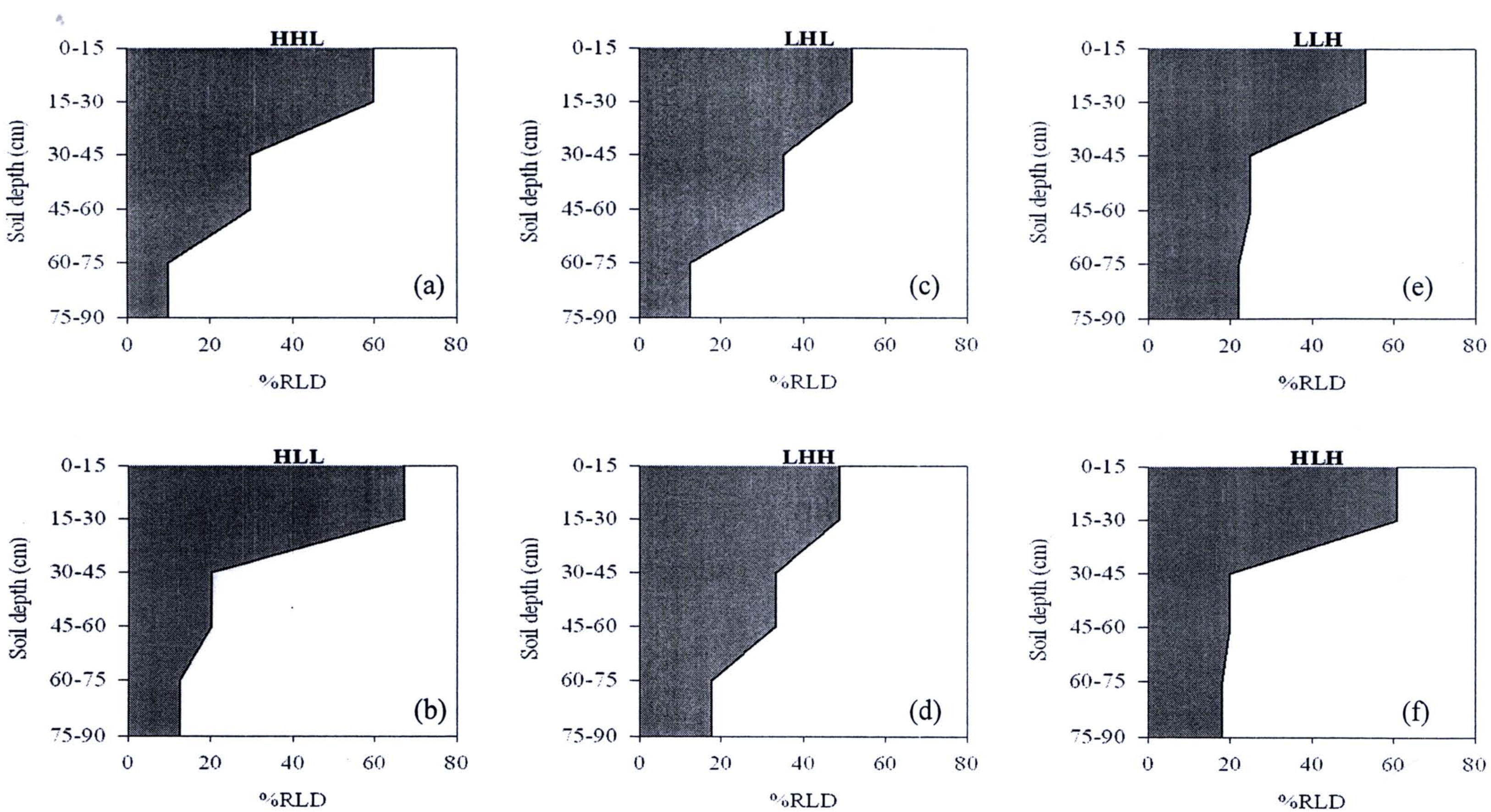

Figure 3 Six root distribution patterns of 40 peanut genotypes (HHL= high RLD in upper and middle layers but low RLD in lower layer (a), HLL= high RLD at upper but low RLD at middle and lower layers (b), LHL= low RLD in upper and lower layers but high RLD in middle layer (c), LHH= low RLD in upper layer but high RLD in middle and lower layers (d), LLH= low RLD in upper and middle layers but high RLD in lower layer (e), HLH= high RLD in upper and lower layers but low RLD in middle layer (f)) from the experiment conducting during December 2007- May 2008 and during November 2008- April 2009 at Khon Kaen University, Khon Kaen, Thailand.

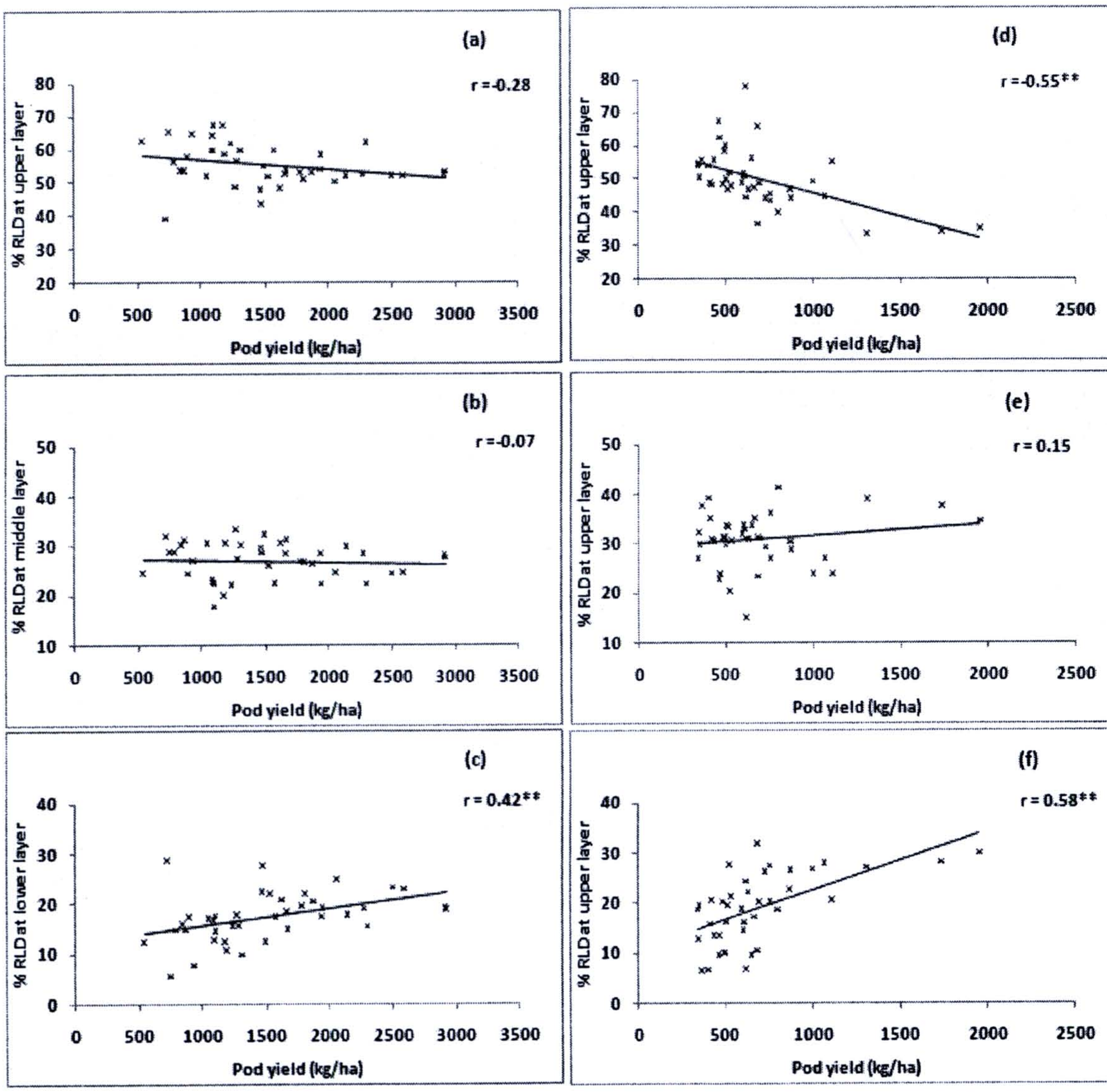

Figure 4 Relationship between pod yield and % root length density (%RLD) three layers as upper middle and lower in the first season (December 2007- May 2008) (a, b and c respectively) and the second season (November 2008- April 2009) (d, e and f respectively) at Khon Kaen University, Khon Kaen, Thailand.

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Table 1 Forty peanut genotypes diverse source countries and drought tolerance levels.

no entries	identification	source contries	drought tolerance levels	no entries	identification	source contries	drought tolerance levels
1	306 PI 430237	USDA[4]	Susceptible	21	ICGV 98300	ICRISAT[1]	drought tolerance
2	12 PI 430233	USDA[4]	Susceptible	22	ICGV 98303	ICRISAT[1]	drought tolerance
3	187 PI 433352	USDA[4]	Susceptible	23	ICGV 98305	ICRISAT[1]	drought tolerance
4	283 PI 234375	USDA[4]	moderate tolerance	24	ICGV 98308	ICRISAT[1]	drought tolerance
5	204 PI 442572	USDA[4]	moderate tolerance	25	ICGV 98324	ICRISAT[1]	drought tolerance
6	5 PI 313160	USDA[4]	moderate tolerance	26	ICGV 98330	ICRISAT[1]	drought tolerance
7	101 PI 268659	USDA[4]	drought tolerance	27	ICGV 98348	ICRISAT[1]	drought tolerance
8	269 PI 157542	USDA[4]	drought tolerance	28	ICGV 98353	ICRISAT[1]	drought tolerance
9	89 PI 157549	USDA[4]	drought tolerance	29	Tifton – 8	USDA[4]	drought tolerance
10	KKU 40	KKU[3] (Thailand)	Unknown	30	248 Grif 13911	USDA[4]	moderate tolerance
11	TAINAN 9	KKFCRC[2] (Thailand)	Susceptible	31	35 Grif 13932	USDA[4]	moderate tolerance
12	KK 5	KKFCRC[2] (Thailand)	Unknown	32	97 PI 158854	USDA[4]	Susceptible
13	KKU 1	KKU[3] (Thailand)	Unknown	33	100 PI 162604	USDA[4]	moderate tolerance
14	KK60-2	KKFCRC[2]and KKU[3] (Thailand)	Unknown	34	102 PI 268660	USDA[4]	drought tolerance
15	KS2	Thailand	Susceptible	35	106 PI 268949	USDA[4]	moderate tolerance
16	KK 4	KKFCRC[2] (Thailand)	Susceptible	36	3 PI 313157	USDA[4]	Susceptible
17	KK60-3	KKFCRC[2]and KKU[3] (Thailand)	Susceptible	37	303 PI 430230	USDA[4]	Susceptible
18	KKU 72-1	KKU[3] (Thailand)	Unknown	38	Taiwan 1	China	Unknown
19	KKU 60	KKU[3] (Thailand)	moderate tolerance	39	Taiwan 2	China	Unknown
20	KK 6	KKFCRC[2] (Thailand)	Unknown	40	Luhua 11	China	Unknown

1 ICRISAT = International Crop Research Institute for the Semi-Arid Tropics

2 KKFC = Khon Kean Field Crop Research Centre

3 KKU = Khon Kean University

4 USDA = United State Department of Agriculture

Table 2 The range of root length density percentage (%RLD) of two amount % RLD group as high and low groups for three layers as upper, middle and lower layers in season1 (December 2007- May 2008) and in season 2 (November 2008- April 2009) at Khon Kaen University, Khon Kaen, Thailand.

Season	Layer	Soil depth (cm)	The range of high %RLD genotypes in each layer	The range of low %RLD genotypes in each layer
Season 1	Upper layer	0-30	67.3-56.1	54.9-39.1
	Middle layer	30-60	33.4-27.2	27.0-17.8
	Lower layer	60-90	28.7-17.4	17.0-5.6
Season 2	Upper layer	0-30	77.8-50.5	49.8-33.5
	Middle layer	30-60	41.1-31.1	30.7-15.1
	Lower layer	60-90	32.0-19.5	19.0-6.5

Table 3 Classification of 33 peanut genotypes for six root distribution patterns (HHL= high RLD in upper and middle layers but low RLD in lower layer (a), HLL= high RLD at upper but low RLD at middle and lower layers (b), LHL= low RLD in upper and lower layers but high RLD in middle layer (c), LHH= low RLD in upper layer but high RLD in middle and lower layers (d), LLH= low RLD in upper and middle layers but high RLD in lower layer (e), HLH= high RLD in upper and lower layers but low RLD in middle layer (f)) from the experiment conducting during December 2007- May 2008 and during November 2008- April 2009 at Khon Kaen University, Khon Kaen, Thailand.

Patterns	**Peanut lines**				
HHL	Tainan 9	KK60-2	KS2	KK 4	35 Grif 13932
HLL	306 PI 430237	248 Grif 13911	97 PI 158854	303 PI 430230	
LHL	187 PI 433352	283 PI 234375	KKU 40	ICGV 98305	100 PI 162604
LHH	101 PI 268659	89 PI 157549	KK60-3	KKU 60	ICGV 98300
	ICGV 98330	102 PI 268660	Taiwan 2	Luhua 11	
LLH	269 PI 157542	KKU 72-1	ICGV 98324	ICGV 98348	106 PI 268949
	Taiwan 1	Tifton – 8			
HLH	ICGV 98353	204 PI 442572			

Table 4 pod yield (PY), top dry weight at 83 day after sowing (TDW at 83 DAS), top dry weight at harvest (TDW at harvest) and pod harvest index (PHI) of the experiment conducting during December 2007- May 2008 at Khon Kaen University, Khon Kaen, Thailand.

line	PY (kg/ha)	TDW at 83 DAS (kg/ha)	TDW at harvest (kg/ha)	PHI
KKU 60	2923 a	3348 a-d	7702 ab	0.270 a
Luhua 11	2913 a	2941 a-f	7286 a	0.279 ab
Taiwan 1	2587 ab	3422 abc	7441 a-h	0.228 abc
KKU 72-1	2499 abc	3058 a-d	6988 abc	0.256 a-d
Tifton – 8	2302 a-d	2617 a-f	6352 a-e	0.248 a-e
35 Grif 13932	2275 a-e	3557 ab	7265 a-g	0.235 a-d
KK 5	2142 a-f	2956 a-f	6530 a-e	0.244 a-e
106 PI 268949	2057 a-f	2641 a-f	6130 a-d	0.250 a-e
KK60-3	1932 a-g	2427 a-f	5791 a-g	0.232 b-g
101 PI 268659	1799 b-h	3020 a-f	6250 a-i	0.223 a-g
269 PI 157542	1776 b-h	2873 a-f	6080 a-j	0.220 a-g
ICGV 98353	1664 b-i	2693 a-f	5789 b-l	0.197 b-g
ICGV 98305	1659 b-i	2802 a-f	5893 a-j	0.219 b-g
89 PI 157549	1655 b-i	2638 a-f	5724 a-k	0.209 b-g
KK 6	1620 b-i	2214 a-f	5821 a-l	0.218 b-g
Taiwan 2	1615 b-i	2955 a-f	6002 a-k	0.199 d-g
204 PI 442572	1568 b-i	2504 b-f	5504 c-l	0.215 d-h
ICGV 98324	1518 c-i	2417 b-f	5368 a-k	0.188 d-h
12 PI 430233	1486 c-i	2288 a-f	5206 a-l	0.206 c-g
KKU 1	1465 c-i	2850 a-f	5747 c-l	0.198 d-h
ICGV 98330	1461 c-i	2596 b-f	5489 a-k	0.180 d-h
KS2	1437 d-i	2407 b-f	5275 a-k	0.214 e-h
97 PI 158854	1353 d-i	2274 b-f	5060 a-l	0.207 e-h
KK60-2	1304 d-i	2144 a-f	4880 c-l	0.201 d-h
KKU 40	1278 d-i	3030 a-f	5740 b-l	0.180 d-h
ICGV 98348	1270 d-i	2715 a-f	5417 b-l	0.190 d-h
102 PI 268660	1268 d-i	2688 ef	5389 a-k	0.188 e-h
ICGV 98303	1234 e-i	1888 a-f	4554 d-l	0.204 d-h
ICGV 98308	1177 f-i	2907 c-f	5515 b-l	0.168 fgh
3 PI 313157	1099 f-i	2010 a-f	4541 e-l	0.190 e-h
248 Grif 13911	1095 f-i	2919 a-f	5446 e-l	0.167 e-h
303 PI 430230	1089 f-i	2483 a	5004 g-l	0.165 b-g
5 PI 313160	1046 ghi	3843 b-f	6321 g-l	0.156 fgh
KK 4	895 ghi	3118 a-e	5444 jkl	0.139 e-h
187 PI 433352	864 ghi	1977 def	4273 f-l	0.164 gh
283 PI 234375	837 ghi	2459 a-f	4728 i-l	0.143 fgh
100 PI 162604	789 hi	2378 b-f	4599 h-l	0.147 fgh
Tainan 9	749 hi	2421 a-f	4602 kl	0.136 fgh
ICGV 98300	719 hi	3016 a-f	5167 l	0.121 e-h
306 PI 430237	538 i	1605 f	3575 l	0.120 h
F-test	**	*	**	**
Mean	1524	2677	5647	0.198
CV (%)	31.6	21.5	20.5	23.8

Mean in the same column with the same letters are not significantly different Dancan's multiple range test (DMRT).
*,** Significant at the 0.05 and 0.01 probability levels, respectively.
CV = coefficient of variation.

Table 5 pod yield (PY), top dry weight at 87 day after sowing (TDW at 87 DAS), top dry weight at harvest (TDW at harvest) and pod harvest index (PHI) of the experiment conducting during November 2008- April 2009 at Khon Kaen University, Khon Kaen, Thailand.

line	PY (kg/ha)	TDW at 87 DAS (kg/ha)	TDW at harvest (kg/ha)	PHI
Luhua 11	1751 a	1390 b-f	3200 a	0.353 bc
KKU 60	1536 a	1586 a-d	5213 bc	0.234 a
KK 6	1102 b	1975 ab	3462 b	0.245 c-e
ICGV 98353	905 bc	1514 a-e	4941 e-g	0.155 ab
Taiwan 1	862 bcd	1956 a	3259 bcd	0.206 c-h
ICGV 98348	797 b-e	1122 d-j	3932 def	0.170 bcd
KKU 72-1	646 c-f	1317 c-g	3317 def	0.161 c-i
ICGV 98305	602 c-g	1342 b-g	3770 d-h	0.138 c-f
ICGV 98330	559 c-h	888 f-j	2968 e-g	0.153 c-k
Tifton – 8	557 c-h	1053 d-j	4508 e-j	0.113 bc
ICGV 98324	528 c-h	1267 d-g	3429 d-h	0.133 c-i
ICGV 98303	496 d-h	1229 d-i	3638 e-j	0.118 c-h
106 PI 268949	490 d-h	810 g-j	3324 e-j	0.130 c-j
KKU 1	488 d-h	1050 d-j	2625 e-g	0.153 d-k
101 PI 268659	484 d-h	1834 abc	4286 e-j	0.103 bcd
KKU 40	471 d-h	1408 b-f	3267 e-i	0.124 c-k
KK60-2	453 e-h	965 f-j	2117 cde	0.175 h-k
ICGV 98308	433 e-h	1001 e-j	3908 e-j	0.098 c-g
204 PI 442572	419 e-h	1023 e-j	3015 e-j	0.124 c-k
KK 5	417 e-h	860 f-j	3273 e-j	0.111 c-k
ICGV 98300	410 e-h	1314 c-g	3772 e-j	0.098 c-h
Taiwan 2	406 e-h	1118 d-j	2837 e-i	0.127 d-k
KK60-3	396 e-h	980 e-j	3382 e-j	0.103 c-k
269 PI 157542	332 fgh	735 hij	2131 e-i	0.131 h-k
248 Grif 13911	325 fgh	937 f-j	2807 e-j	0.102 d-k
12 PI 430233	323 fgh	1041 d-j	2526 e-j	0.114 f-k
102 PI 268660	313 fgh	1075 d-j	3471 f-j	0.099 c-k
3 PI 313157	303 fgh	1177 d-j	2457 e-j	0.099 f-k
KK 4	296 fgh	1019 e-j	2708 e-j	0.097 e-k
187 PI 433352	289 fgh	1025 e-j	2206 e-j	0.117 h-k
303 PI 430230	269 fgh	1086 d-j	2304 e-j	0.106 h-k
306 PI 430237	264 fgh	740 hij	1960 e-i	0.120 jk
Tainan 9	234 fgh	1047 d-j	2774 g-j	0.074 e-k
89 PI 157549	221 fgh	940 f-j	2052 e-j	0.095 ijk
100 PI 162604	211 gh	682 ij	2581 g-j	0.075 f-k
KS2	203 gh	613 j	1976 f-j	0.095 jk
35 Grif 13932	166 h	1020 e-j	1925 f-j	0.089 k
5 PI 313160	153 h	919 f-j	2502 hij	0.059 g-k
97 PI 158854	147 h	1162 d-j	3472 j	0.039 c-k
283 PI 234375	140 h	893 f-j	2582 ij	0.053 f-k
F-test	**	**	**	**
Mean	484	1127	3096	0.127
CV (%)	38.0	22.4	26.9	27.3

Mean in the same column with the same letters are not significantly different Dancan's multiple range test (DMRT).
** Significant at the 0.01 probability levels,
CV = coefficient of variation.

Table 6 Correlation coefficients (r) (n = 40) between top dry weight (TDW) at 83 DAS, TDW at harvest, pod harvest index (PHI) and pod yield (PY) three layers as upper middle and lower in season1 (December 2007- May 2008) and TDW at 87 DAS, TDW at harvest, PHI and PY in season 2 (November 2008- April 2009) at Khon Kaen University, Khon Kaen, Thailand.

Season		TDW at 83 DAS (season1) at 87 DAS (season2)	TDW at harvest	PHI
Season 1	PY	0.49**	0.89 **	0.94 **
Season 2	PY	0.51**	0.58 **	0.83 **

** = significant at 1 % level and not significant respectively

Table 7 Correlation coefficients (r) (n = 40) between top dry weight (TDW) at 83 DAS, TDW at harvest, pod harvest index (PHI) and % root length density (%RLD) three layers as upper middle and lower in season1 (December 2007- May 2008) and TDW at 87 DAS, TDW at harvest, PHI and %RLD in season 2 (November 2008- April 2009) at Khon Kaen University, Khon Kaen, Thailand.

Season 1		TDW at 83 DAS	TDW at harvest	PHI
%RLD	upper layer	-0.42 **	-0.39 *	-0.21
%RLD	middle layer	0.14	0.02	-0.12
%RLD	lower layer	0.44 **	0.47 **	0.36 *
Season 2		**TDW at 87 DAS**	**TDW at harvest**	**PHI**
%RLD	upper layer	-0.48 **	-0.43 **	-0.37 *
%RLD	middle layer	0.25	0.01	0.14
%RLD	lower layer	0.42 **	0.55 **	0.36 *

**, * = significant at 1, 5 % level and not significant respectively