



Association of nitrogen fixation to water uses efficiency and yield traits of peanut

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Abstract

Improvement of N₂ fixation might be an effective strategy in peanut breeding for high yield under drought stress conditions. However, under water limited conditions peanut varieties having high water-use efficiency (WUE) are favorable. A pot experiment was conducted under greenhouse conditions at Khon Kaen University, Thailand during December 2002 to May 2003, and repeated during June 2003 to November 2003. Twelve peanut genotypes were tested under three water regimes to estimate the relationships between N₂ fixed with biomass production, WUE and pod yield under drought stress conditions. N₂ fixed biomass production; pod yield and WUE were reduced by drought stress. At 2/3 AW, Tifton-8 and KK 60-3 were the best genotypes for high N₂ fixed and high WUE. ICGV 98324 and ICGV 98300 had high pod yield, whereas Tifton-8 had low pod yield. N₂ fixed was positively correlated with biomass and WUE under mild drought conditions but negatively correlated with pod yield. Tifton-8 was the best genotype for N₂ fixed and WUE, but it was a poor performer for pod yield under drought conditions. ICGV 98324 and ICGV 98300 had higher pod yield with lower N₂ fixed and WUE than did Tifton-8. Results indicated that N₂ fixed under drought conditions contributed to vegetative growth and water use efficiency rather than to pod yield. Improvement for high N₂ fixed in peanut could lead to high biomass production and WUE but may not necessarily improve pod yield under drought stress conditions.

Keywords: *Arachis hypogaea* L.; Biomass production; Drought conditions; Drought resistance; Pod yield.

Introduction

Drought is a recurring problem limiting peanut yield in rain-fed areas of the semi-arid tropics (Wright et al., 1991; Wright and Nageswara Rao, 1994; Nautiyal et al., 1999; Reddy et al., 2003) and it also reduces N₂ fixation (Venkateswarlu et al., 1990; Peoples et al., 1992; Sinclair et al., 1995; Serraj et al., 1999; Hungria and Vargas, 2000; Giller, 2001; Thomas et al., 2004). Although access to irrigation can be used to alleviate drought, it is limited for most peanut production areas and the most promising strategy is to use drought resistant varieties.

Symbiotic nitrogen fixation is important for growth and yield of leguminous crops especially in infertile soils. Under drought stress conditions, the available soil N is greatly reduced. As nitrogen is an essential element for growth and yield of the crop, such reduction in the available soil N leads to low N accumulation and consequently low dry matter production and low crop yield (Chapman and Muchow, 1985; De Vries et al., 1989; De Silva et al., 1996). The genotypes that can fix high N₂ under drought stress conditions might enhance crop productivity under water-limited conditions. Pimratch et al. (2008) reported that the ability to maintain high N₂ fixation under drought stress could aid peanut genotypes in maintaining high yield under water limited conditions. Improvement of N₂ fixation might be an effective strategy in breeding peanut for high yield under drought stress conditions.

Several authors have reported that dry matter partitioning is very important in the determination of crop yield (Kumari and Singh, 1990; Bell et al., 1994). Total biomass has been used as a selection criterion for assessing drought resistance in peanut (Nageswara Rao et al., 1994). The drought resistant lines identified through this process are those that perform well and give high yields under drought conditions (Nageswara Rao et al., 1994; Nigam et al., 2003; Nigam et al., 2005). Drought resistance can be enhanced by improving the ability to produce biomass per unit water use. This might be achieved by selection for high water use efficiency (WUE). Development of peanut varieties resistant to drought and efficient in water use offers the best long term and cost effective solution to the uncertainty of availability of water.

The peanut genotypes with high nitrogen fixation might contribute to high WUE and enhanced high crop productivity under water limited conditions. However, information on the relationship between nitrogen

fixation and water-use efficiency in peanut under drought conditions is lacking. Whether or not higher nitrogen fixation will contribute to higher WUE under drought stress needs to be established in peanut. A better understanding on the relationship between N₂ fixation and WUE under water stress conditions should have implications in breeding peanut for high N₂ fixation under drought stress.

The objective of this study was to estimate relationships between N₂ fixed with biomass production, WUE and pod yield under drought stress conditions.

Materials and Methods

Experimental design

A pot experiment was conducted under greenhouse conditions at the Field Crop Research Station of Khon Kaen University located in Khon Kaen province (latitude 16° 28' N, longitude 102° 48' E, 200 m above sea level) during December 2002 to May 2003 and repeated during June 2003 to November 2003. A₃×12 factorial combination in RCBD with 6 replications was used for both experiments. Three soil moisture levels [field capacity (FC), 2/3 available soil water (2/3 AW) and 1/3 available soil water (1/3 AW)] were assigned as factor A and 12 peanut genotypes as factor B. The soil on the experimental site pertains to the Yasothon series (Y_i; fine-loamy, siliceous, isohythermic, Oxic Paleustults). The proportions of sand, silt and clay in the soil were 56.84%, 24.79% and 18.37%, respectively. The soil is sandy loam with pH 5.20, 0.196% organic matter and 0.0093% total N. Available P was 4.88 ppm (Bray II method) and extractable K and Ca were 49.55 and 444.94 ppm, respectively.

Twelve peanut genotypes were used in this study. Eight (ICGV 98300, ICGV 98303, ICGV 98305, ICGV 98308, ICGV 98324, ICGV 98330, ICGV 98348 and ICGV 98353) were elite drought resistant lines obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India, one accession (Tifton-8) is a Virginia-type drought resistant line (Coffelt et al., 1985) received from the United State Department of Agriculture (USDA), two (KK 60-3 and Tainan 9) are released cultivars commonly grown in Thailand and one is a non-nodulating line (Non-nod) included as reference plant in determining nitrogen fixation. The lines from ICRISAT were identified as drought resistant because they had

high total biomass and pod yield in screening tests under drought stress conditions (Nageswara Rao et al., 1994; Nigam et al., 2003; Nigam et al., 2005). KK 60-3 is a Virginia-type peanut cultivar with high N₂ fixation (Toomsan et al., 1995) but it is sensitive to drought for pod yield, while Tainan 9 is a Spanish-type peanut cultivar having low dry matter production (Vorasoet et al., 2003) and low N₂ fixation (Mc Donagh et al., 1993).

Crop management

Pots with 25 cm in diameter and 70 cm in height were used. Each pot was filled to 10 cm from the top with 42 kg dry soil to create uniform bulk density. Each treatment consisted of 2 pots in a replicate. Seeds were treated with captan (3a,4,7,7a-tetrahydro-2-[(trichloromethyl) thio]-1H-isindole-1,3(2H)-dione) at the rate of 5 g/kg seed before planting and seeds of the two Virginia-type peanut genotypes (KK 60-3 and Tifton-8) were also treated with ethrel 48% at the rate of 2 ml L⁻¹ water to break dormancy. A commercial peat-based inoculum of *Bradyrhizobium* (mixture of strains THA 201 and THA 205; Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand) was applied with the seed at planting. Seed was planted 3 seeds pot⁻¹ and plants were then thinned to 2 plants pot⁻¹ at 14 days after emergence (14 DAE). Phosphorus fertilizer as triple super phosphate (Ca(H₂PO₄)₂.H₂O) at the rate of 12.12 g P pot⁻¹ and potassium fertilizer as muriate of potash (KCl) at 15.26 g Kpot⁻¹ were applied at 14 DAE. Gypsum (CaSO₄) at the rate of 153.08 g pot⁻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] at 2.5 Lha⁻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 kgha⁻¹.

The water supplied to individual pots was equal to the sum of water used by the crop and soil surface evaporation. The calculated amount of water was divided into four fractions. The first fraction was applied on the soil surface and the three fractions were loaded in three cones to supply water to the soil columns through plastic tubes at 25, 40 and 55 cm below the top of the pots, respectively. Soil water level was maintained uniformly at field capacity

(17.81%) from planting to 14 DAE and then soil moistures of stress treatments were allowed to gradually reduce until they reached predetermined levels of 2/3 AW and 1/3 AW stress treatments at 21 and 28 DAE, respectively. The soil moisture was then held at these levels until harvest.

Soil moisture at FC and permanent wilting point (PWP) were determined as 17.81% and 6.8%, respectively, using pressure plate method. Soil moisture content for 2/3 and 1/3 AW treatment was estimated to be 14.14% and 10.47% as a proportional value between FC and PWP. For each treatment, moisture content was maintained uniformly with no more than 1% moisture change of the predetermined levels until harvest. In maintaining the specified soil moisture levels, water was added to the respective pots 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).

Data Collection

Weather data, soil moisture and plant water status

Rain fall, relative humidity, evaporation, maximum and minimum temperature and solar radiation were recorded daily from sowing until harvest by a weather station near the greenhouse. Soil moisture was measured by the gravimetric method before planting and at harvest for both seasons. Briefly, a sample was taken from each pot using a soil sampler through the whole column and mixed thoroughly. The small portion of the soil sample was oven-dried and percent moisture was determined.

Leaf water potential (LWP) and relative water content (RWC) were measured at 30, 60 and 90 DAE to evaluate plant water status from the first pot. A pressure bomb model 1003 S/N 2973 ("PMS" Pressure bomb) was used to determine LWP using the third leaf from the top of the main stem sampled from one plant in each pot at 10.00-12.00 AM. RWC was measured following Kramer (1980), using the second leaf from the top of the main stem sampled from one plant in each pot. RWC was calculated as:

$$\text{RWC} = \left[\frac{\text{fresh weight} - \text{dry weight}}{\text{saturated weight} - \text{dry weight}} \right] \times 100$$

Saturated weight was determined by putting the leaf sample in water for 8 hours; blot drying the outer surface and then measuring leaf weight.

N₂-fixation, biomass production, pod yield and water use efficiency

For each treatment, plants in the second pot were uprooted and soil was gently removed from the root by washing them on a 0.5 mm screen. Nodules were then removed from each root by hand and counted. The nodules, root, shoot and pod were oven-dried at 75 °C for 48 hours and weighed. Biomass production (total dry weight) consisted of root, nodule, shoot and pod dry weight.

Fixed nitrogen was determined after harvest by the N-difference method using the non-nodulating line as reference plant. Samples were taken from shoots and analyzed for crude protein using micro-kjedahl method (Black, 1965). Total nitrogen was then determined using the automated indophenol method (Schuman et al., 1973) and read on a flow injection analyzer model 5012 (Tecator Inc., Hoganas, Sweden). Fixed nitrogen contents were calculated as:

$$\text{Fixed N}_2 \text{ of each genotype} = (\text{Total N of each genotype}) - (\text{Total N of non - nodulating line})$$

The N-difference method using the non-nodulating line as reference plant was selected for this study because it is reliable and economical. This method has been proven in previous studies to be as effective as the ¹⁵N isotope dilution method in determining nitrogen fixation (Mc Donagh et al., 1993; Bell et al., 1994; Phoomthaisong et al., 2003).

Water use efficiency (WUE) for each treatment was calculated as biomass production divided by water use.

$$\text{WUE} = \text{Biomass production (g)} / \text{Water use (kg)}$$

Statistical analysis

Individual analysis of variance was performed for each character in each season. Error variances for the two seasons were tested for homogeneity by Bartlett's test (Hoshmand, 2006). Combined analyses of variance were done for those characters that had homogeneous error variances for the two seasons. Least Significant Difference (LSD) was used to compare means.

The analyses of variance at this stage were done using MSTAT-C package (Bricker, 1989). Simple correlation was used to determine the relationship between fixed nitrogen with biomass production, pod yield and WUE under well-watered and drought stress conditions.

Results

Weather data, soil moisture and plant water status

The seasonal maximum and minimum air temperature ranged between 33.4 °C and 21.8 °C in greenhouse 1 (GH₁) and 34.5 °C and 24.3 °C in greenhouse 2 (GH₂). Daily pan evaporation ranged from 2.1 to 9.4 mm in GH₁ and 0.1 to 10.1 mm in GH₂. The seasonal mean relative humidity was 81.3% in GH₁ and 90.7% in GH₂. The seasonal means of solar radiation were 19.2 MJm⁻²d⁻¹ in GH₁ and 15.7 MJm⁻²d⁻¹ in GH₂.

Prior to initiation of experiments, soils were analyzed by pressure plate method to determine water holding capacity of the soils. At field capacity, the soil water holding capacity was 17.81% and permanent wilting point was 6.80%. Therefore, soil water holding capacities at 2/3 AW and 1/3 AW were determined to be 14.14% and 10.47%, respectively. Soil moistures of the three water regimes measured at harvest were 16.72%, 14.28% and 9.21% for FC, 2/3 AW and 1/3 AW, respectively, for GH₁ and 16.74%, 13.22% and 10.40% for FC, 2/3 AW and 1/3 AW, respectively, for GH₂. The soil moistures measured were close to desired levels of 17.81%, 14.14% and 10.47% for FC, 2/3 AW and 1/3 AW, respectively, indicating appropriate control of the treatments.

LWP and RWC were significantly lower in the stressed treatments than the control (data not shown). The highest LWP and RWC were observed for soil moisture contents at field capacity (FC) followed by 2/3 AW and 1/3 AW, respectively. Plants in the 1/3 AW treatment also showed more severe wilting than did the plants in the 2/3 AW treatment in the afternoon, whereas plants in field capacity treatment were normal.

Water stress effects on N₂ fixation, biomass production, WUE and pod dry weight

Data of two seasons were combined because the interaction effects for season×genotype (S×G) were relatively low for nitrogen fixation and pod

dry weight compared to genotype (G) main effects (data not shown) and not significant for biomass production and water use efficiency (WUE).

The results clearly showed that drought stress reduced N₂ fixed, biomass production (Table 1), WUE and pod dry weight (Table 2). Significant differences among peanut genotypes were found for all traits under different water regimes. Under well-watered conditions, Tifton-8 had the highest N₂ fixation followed by the genotypes ICGV 98330, ICGV 98303 and ICGV 98300. Tifton-8 and KK 60-3 were the best genotypes for high N₂ fixed under drought conditions at both 2/3 AW and 1/3 AW. Tifton-8, KK 60-3 and ICGV 98300 were the best genotypes for biomass production under well-watered conditions and 2/3 AW. At 1/3 AW, ICGV 98300 and Tifton-8 also had the highest biomass production followed by ICGV 98330 and ICGV 98324. Similar to the biomass production, Tifton-8, ICGV 98300 and KK 60-3 had high WUE under all well-watered conditions and both drought stress conditions. KK 60-3 and ICGV 98300 had the highest pod dry weight under well-watered conditions. At 2/3 AW, ICGV 98300 and ICGV 98324 had high pod yield, whereas Tifton-8 has low pod yield. ICGV 98324 was the best genotype for pod yield at 1/3 AW.

Table 1. N₂ fixation (mg N plant⁻¹) and biomass production (g plant⁻¹) of 11 peanut genotypes (excluded non-nodulating line) grown under different water regimes.

| Genotypes | N ₂ fixation | | | Biomass production | | |
|------------|-------------------------|----------------------------|--------------|--------------------|--------------|-------------|
| | FC | 2/3 AW | 1/3 AW | FC | 2/3 AW | 1/3 AW |
| ICGV 98300 | 122.47 | 49.86 (59.3) ^{1/} | 39.19 (68.0) | 20.60 | 12.64 (38.6) | 9.42 (54.3) |
| ICGV 98303 | 130.53 | 59.31 (54.6) | 47.70 (63.5) | 17.93 | 11.23 (37.4) | 8.09 (54.9) |
| ICGV 98305 | 104.12 | 53.00 (49.1) | 43.44 (58.3) | 18.30 | 11.51 (37.1) | 7.89 (56.9) |
| ICGV 98308 | 103.08 | 47.81 (53.6) | 33.78 (67.2) | 18.52 | 11.53 (37.7) | 8.25 (55.5) |
| ICGV 98324 | 101.97 | 45.43 (55.4) | 46.20 (54.7) | 17.85 | 11.80 (33.9) | 9.00 (49.6) |
| ICGV 98330 | 145.43 | 55.79 (61.6) | 51.30 (64.7) | 19.51 | 10.73 (45.0) | 9.06 (53.6) |
| ICGV 98348 | 118.43 | 56.87 (52.0) | 38.73 (67.3) | 19.26 | 11.56 (40.0) | 7.85 (59.2) |
| ICGV 98353 | 90.08 | 51.19 (43.2) | 27.58 (69.4) | 17.64 | 11.03 (35.7) | 8.09 (54.1) |
| Tainan 9 | 90.16 | 57.36 (36.4) | 43.23 (52.1) | 17.35 | 11.24 (35.2) | 8.22 (52.6) |
| KK 60-3 | 111.23 | 100.87 (9.3) | 65.74 (40.9) | 21.52 | 12.02 (44.1) | 8.25 (61.7) |
| Tifton -8 | 189.71 | 144.63 (23.8) | 80.55 (57.5) | 23.85 | 13.21 (44.6) | 9.26 (61.2) |
| Mean | 118.84 | 65.65 (45.3) | 47.04 (60.3) | 19.30 | 11.68 (39.0) | 8.49 (55.7) |
| LSD (0.05) | 31.31 | 17.89 | 13.58 | 1.54 | 1.16 | 0.88 |
| C.V. (%) | 35.60 | 33.63 | 32.51 | 12.82 | 12.24 | 9.81 |

FC, field capacity; AW, available soil water.

^{1/} The numbers in parenthesis are reduction percentage.

Table 2. Water use efficiency (g kg^{-1}) and pod dry weight (g plant^{-1}) of 11 peanut genotypes (excluded non-nodulating line) grown under different water regimes.

| Genotypes | Water use efficiency | | | Pod dry weight | | |
|------------|----------------------|---------------------------|-------------|----------------|-------------|-------------|
| | FC | 2/3 AW | 1/3 AW | FC | 2/3 AW | 1/3 AW |
| ICGV 98300 | 1.69 | 1.25 (26.0) ^{1/} | 1.18 (30.2) | 8.60 | 4.05 (52.9) | 1.05 (88.7) |
| ICGV 98303 | 1.46 | 1.16 (20.5) | 1.01 (30.8) | 6.81 | 3.04 (55.4) | 0.90 (86.8) |
| ICGV 98305 | 1.47 | 1.15 (21.8) | 0.98 (33.3) | 7.30 | 3.29 (54.9) | 0.93 (87.3) |
| ICGV 98308 | 1.50 | 1.16 (22.7) | 1.03 (31.1) | 5.85 | 2.96 (49.4) | 1.05 (82.1) |
| ICGV 98324 | 1.45 | 1.18 (18.6) | 1.13 (22.1) | 6.92 | 4.00 (42.2) | 1.96 (71.1) |
| ICGV 98330 | 1.57 | 1.07 (31.8) | 1.10 (29.9) | 7.35 | 3.12 (57.6) | 1.08 (85.3) |
| ICGV 98348 | 1.55 | 1.14 (26.5) | 0.98 (36.8) | 7.04 | 3.30 (53.1) | 1.08 (84.7) |
| ICGV 98353 | 1.44 | 1.11 (22.9) | 1.01 (29.9) | 6.98 | 3.38 (51.6) | 1.06 (84.8) |
| Tainan 9 | 1.41 | 1.13 (19.9) | 1.02 (27.7) | 7.16 | 3.12 (56.4) | 0.90 (87.4) |
| KK 60-3 | 1.52 | 1.20 (21.1) | 1.14 (25.0) | 9.25 | 2.52 (72.8) | 0.43 (95.5) |
| Tifton -8 | 1.69 | 1.33 (21.3) | 1.15 (32.0) | 6.38 | 0.75 (88.2) | 0.03 (99.5) |
| Mean | 1.52 | 1.17 (23.0) | 1.07 (29.9) | 7.24 | 3.05 (57.7) | 0.95 (86.7) |
| LSD (0.05) | 0.11 | 0.11 | 0.16 | 1.05 | 0.73 | 0.47 |
| C.V. (%) | 18.03 | 11.75 | 9.16 | 16.90 | 20.02 | 17.84 |

FC, field capacity; AW, available soil water.

^{1/} The numbers in parenthesis are reduction percentage.

Relationship between N₂ fixation with biomass production, WUE and pod dry weight

Positive relationships between N₂ fixed and biomass production were found at FC ($r=0.79$, $P=0.01$) (Figure 1a) and drought stress at 2/3 AW ($r=0.67$, $P=0.05$) (Figure 1b). Tifton-8 had the highest N₂ fixed and biomass production at all water regimes. The correlation coefficients between N₂ fixed and WUE under well-watered conditions ($r=0.76$, $P=0.01$) (Figure 2a) and drought stress at 2/3 AW ($r=0.71$, $P=0.05$) (Figure 2b) were also positive and significant. However, the correlation coefficients of these traits were not significant at 1/3 AW ($r=0.56$) (Figure 2c). The relationships between N fixed and WUE were similar to those between N fixed and biomass production and the genotype with high N₂ fixed had high WUE especially for Tifton-8.

There were significant and reverse correlations between N₂ fixed and pod yield, especially at 2/3 AW ($r=-0.92$, $P=0.01$) (Figure 3b) and at 1/3 AW ($r=-0.66$, $P=0.05$) (Figure 3c), where as the correlation was not significant under well-watered conditions ($r=-0.31$) (Figure 1a). The genotypes with high N₂ fixed had low pod yield. Tifton-8 and KK 60-3 were the best genotypes for N₂ fixed but they had low pod yield under both drought conditions. At 2/3 AW, ICGV 98324 and ICGV 98300 had higher pod yield but they had lower N₂ fixed than did Tifton-8 and KK 60-3. At 1/3 AW, ICGV 98324 had high pod yield and moderate N₂ fixed.

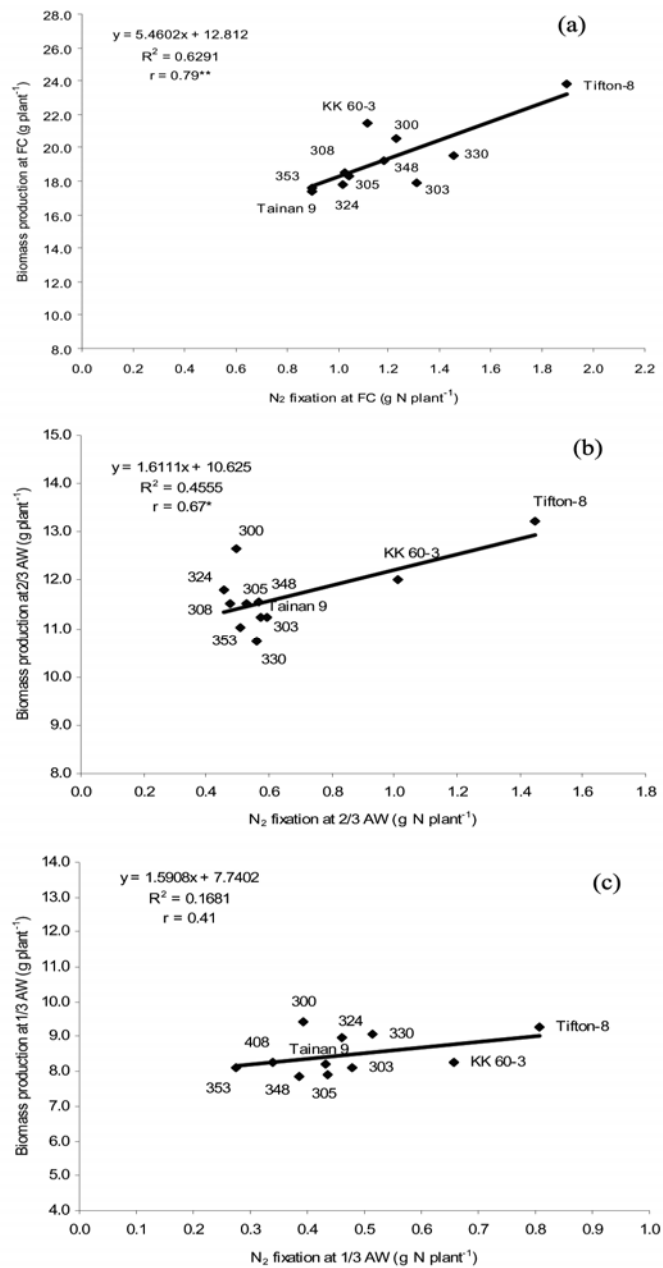


Figure 1. Relationship between N_2 fixation and biomass production at field capacity (FC) (a), 2/3 available soil water (AW) (b) and 1/3 AW (c) of 11 peanut genotypes (excluded non-nodulating line).

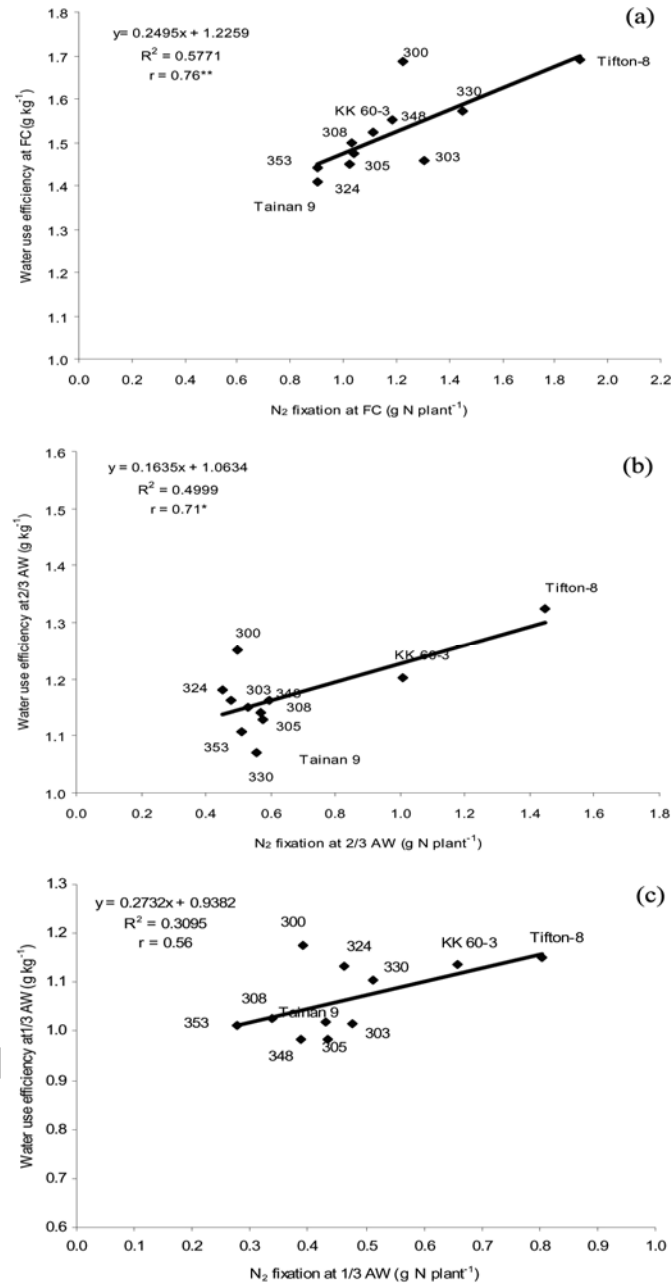


Figure 2. Relationship between N_2 fixation and water use efficiency (WUE) at field capacity (FC) (a), 2/3 available soil water (AW) (b) and 1/3 AW (c) of 11 peanut genotypes (excluded non-nodulating line).

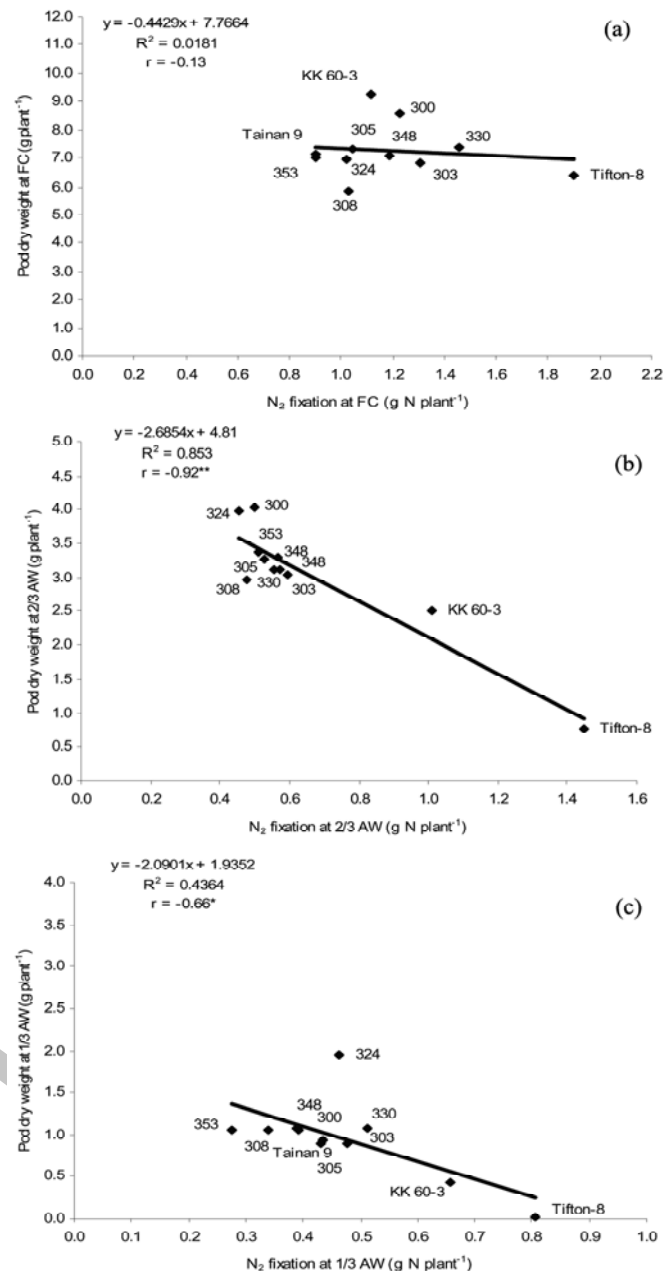


Figure 3. Relationship between N_2 fixation and pod dry weight at field capacity (FC) (a), 2/3 available soil water (AW) (b) and 1/3 AW (c) of 11 peanut genotypes (excluded non-nodulating line).

Discussion

Soil moisture and plant water status were carefully managed by frequent checkup of soil moisture and leaf water potential and the soil moisture contents and leaf water potentials of different water regimes were close to the predetermined levels for both studies. Results revealed that water treatments were adequately controlled in both greenhouses. The results were in agreement with leaf water status and soil moisture contents which were monitored regularly.

As the interactions between peanut genotype and environment were low for all characters, the data from the both studies were combined. The low genotype×water regime interactions also indicated the consistency of the traits under different water regimes. The question underlying the study is whether N_2 fixed contributes to pod yield and water use efficiency of peanut or it merely contributes to biomass production.

Drought stress, in general, reduced nitrogen fixation, biomass production, WUE and pod yield. The more severe the drought stress increased the more reductions in these traits. However, the extents to which drought stress affected the traits were rather different among traits. Pod yield was severely affected by drought stress as early as the moderate drought stress (2/3 AW) and most severe at the severe drought stress (1/3 AW). Biomass production was less affected by drought than pod yield which was similar to nitrogen fixation, whereas water use efficiency had the smallest reduction.

Peanut genotypes showed differential responses for these traits. For example, Tifton 8 and KK 60-3 were very sensitive to drought stress for pod yield and pod yield reduction was faster than reductions in biomass and nitrogen fixation. This may indicate that nitrogen fixation contributes to biomass production rather than to pod yield under drought conditions.

Under drought conditions, N_2 fixed was positively correlated with biomass production and WUE but it was negatively correlated with pod yield. The results indicated that N_2 fixed under drought conditions contributed to vegetative growth and water use efficiency rather than to pod yield. Improvement for high N_2 fixed in peanut might lead to high WUE but it is not necessary to improve pod yield under drought stress conditions. This might be due to limited partitioning of assimilates to the pods.

Therefore, selection for high harvest index as a supplemental trait may be necessary for drought resistance breeding. The competition of assimilate supply between harvestable sink and vegetative sink under different water

levels could alter the relationships of these traits (Vorasoot et al., 2004). Songsri et al. (2009) reported that ICGV 98300 and ICGV 98324 had high HI under drought stress conditions, whereas, Tifton-8 and KK 60-3 were the lowest HI under drought stress conditions. Based on our results, Tifton-8 and KK 60-3 were the best genotypes for high nitrogen fixation, biomass production and WUE under mild drought stress conditions but these genotypes had low pod yield. However, ICGV 98324 and ICGV 98300 had higher pod yield under drought stress but they had lower N_2 fixed than did Tifton-8 and KK 60-3. They showed consistent performance across greenhouses and should be useful as germplasm sources for future crossing programs.

Pimratch et al. (2008) reported that positive relationships between N_2 fixed and biomass production and they suggested that the ability to maintain high N_2 fixation under drought stress could aid peanut genotypes in maintaining high yield and under water limited conditions. In addition, Arunyanark et al. (2012) revealed that the ability to maintain high N_2 fixation under drought conditions can result in better resistance to aflatoxin contamination. Drought resistance in peanut may be enhanced by improving the extraction of water from soil (Wright and Nageswara Rao, 1994). Drought adaptive traits have been identified such as large root systems (Rucker et al., 1995) and high root length density in the lower soil layers (Songsri et al., 2008) that can be used as selection criteria for developing drought resistance. Songsri et al. (2009) reported that the ability of peanut genotypes with large root systems to maintain high WUE under both drought and well-watered conditions.

Nitrogen fixation is useful for growth and yield of peanut. Under drought conditions the results might indicate that the ability of harvestable pods to obtain sufficient assimilates was most difficult because high competition of vegetative sink (Songsri et al., 2009). This could be due to the limited water supply to carry assimilates from source to harvestable sink. Therefore, high nitrogen fixation should contribute more to pod yield under drought conditions if the peanut genotypes can maintain high water uptake (Puangbut et al., 2011)

It has been concluded that nitrogen fixation, biomass production, WUE and pod yield were reduced by drought stress and the reductions of these traits were increased with severity of drought stress. Under drought conditions, N_2 fixed was positively correlated with biomass and WUE but negatively correlated with pod yield. Tifton-8 was the best genotype for N_2 fixed and WUE, but it was poor performer for pod yield under drought conditions.

ICGV 98324 and ICGV 98300 had higher pod yield but they had lower N₂ fixed than did Tifton-8 and KK 60-3. The development of peanut with high nitrogen fixation could enhance high WUE under drought stress conditions.

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References

- Arunyanark, A., Pimratch, S., Jogloy, S., Wongkaew, S., Vorasoot, N., Akkasaeng, C., Kesmala, T., Patanothai, A., Holbrook, C.C., 2012. Association between aflatoxin contamination and N₂ fixation in peanut under drought conditions. *Int. J. Plant Prod.* 6, 161-172.
- Bell, M.J., Wright, G.C., Suryantini, Peoples, M.B., 1994. The N₂-fixing capacity of peanut cultivars with differing assimilate partitioning characteristics. *Aust. J. Agric. Res.* 45, 1455-1468.
- Black, C.A., 1965. Method of Soil Analysis Part 2. Agronomy 9. American of Society of Agronomy, Wisconsin.
- Bricker, A.A., 1989. MSTAT-C User's Guide. Michigan State University, East Lansing, Michigan.
- Chapman, A.L., Muchow, R.C. 1985. Nitrogen accumulated and partitioned at maturity by grain legumes grown under different water regimes in a semi-arid tropical environment. *Field Crops Res.* 11, 69-79.
- Coffelt, T.A., Hammons, R.O., Branch, W.D., Mazingo, R.W., Phipps, P.M., Smith, J.C., Lynch, R.E., Kvien, C.S., Ketring, D.L., Porter, D.M., Mixon, A.C., 1985. Registration of Tifton-8 peanut germplasm. *Crop Sci.* 25, 203.
- DeSilva, M.L., Purcell, C., King, C.A., 1996. Soybean petiole ureide response to water deficits and decreased transpiration. *Crop Sci.* 36, 611-616.
- DeVries, J.D., Bennett, J.M., Albrecht, S.L., Boote, K.J., 1989. Water relations, nitrogenase activity and root development of three grain legumes in response to soil water deficits. *Field Crops Res.* 22, 215-226.
- Doorenbos, J., Pruitt, W.O., 1992. Calculation of crop water requirements. In: Doorenbos, J., Pruitt, W.O. (Eds), *Guideline for Predicting Crop Water Requirements: FAO Irrigation and Drainage Paper No. 24*. FAO, Rome, pp. 1-65.
- Giller, K.E., 2001. *Nitrogen Fixation in Tropical Cropping Systems*. CAB International, Wallingford.

- Hoshmand, A.R., 2006. Design of Experiments for Agriculture and the Natural Sciences, Chapman & Hall: Florida.
- Hungria, M., Vargas, M.A.T., 2000. Environmental factors affecting N₂ fixation in grain legumes in the tropics, with an emphasis on Brazil. *Field Crops Res.* 65, 151-164.
- Kramer, P.J., 1980. Drought, stress and the origin of adaptation. In: N.C. Turner, and P.J. Kramer, eds. *Adaptation of Plant to Water and High Temperature Stress*, John Wiley & Sons, New York. pp. 7-20.
- Kumari, T.S., Singh, B.G., 1990. Analysis of dry matter production and yield potential in groundnut genotypes. *J. Maharashtra Agric. Univ.* 15, 310-313.
- Mc Donagh, J.F., Toomsan, B., Limpinuntana, V., Giller, K.E., 1993. Estimates of the residual nitrogen benefit of groundnut to maize in Northeast Thailand. *Plant Soil*, 154, 267-277.
- Nageswara Rao, R.C., Reddy, L.J., Mehan, V.K., Nigam, S.N., McDonald, D., 1994. Drought research on groundnut at ICRISAT. In: N.S. Nigam, ed. *Proceedings of an International Workshop Groundnut a Global Perspective*, ICRISAT, Patancheru, AP, India, 455p.
- Nautiyal, P.C., Ravindra, V., Zala, P.V., Joshi, Y.C., 1999. Enhancement of yield in groundnut following the imposition of transient soil-moisture stress during the vegetative phase. *Exp. Agric.* 35, 371-385.
- Nigam, S.N., Basu, M.S., Cruickshank, A.W., 2003. Hybridization and description of the trait-based and empirical selection programs. In: Cruickshank, A.W., Rachaputi, N.C., Wright, G.C., Nigam, S.N. (Eds.), *breeding for Drought-resistant Peanuts*. Report of a Workshop held at ICRISAT Centre, Andhra.
- Nigam, S.N., Chandra, S., Rupa Sridevi, K., Manohar Bhukta, Reddy, A.G.S., Nageswara Rao, R.C., Wright, G.C., Reddy, P.V., Deshmukh, M.P., Mathur, R.K., Basu, M.S., Vasundhara, S., Vindhiya Varman, P., Nagda, A.K., 2005. Efficiency of physiological trait-based and empirical selection approaches for drought tolerance in groundnut. *Ann. Appl. Biol.* 146, 433-439.
- Phoomthaisong, J., Toomsan, B., Limpinuntana, V., Cadisch, G., Patanothai, A., 2003. Attributes affecting residual benefits of N₂-fixing mungbean and groundnut cultivars. *Biol. Fert. Soils*, 39, 16-24.
- Peoples, M.B., Bell, V.J., Bushby, H.V.A., 1992. Effect of rotation and inoculation with Bradyrhizobium on nitrogen fixation and yield of peanut (*Arachis hypogaea* L., cv. Virginia Bunch). *Aust. J. Agric. Res.* 43, 595-607.
- Pimratch, S., Jogloy, S., Vorasoot, N., Toomsan, B., Patanothai, A., Holbrook, C.C., 2008. Relationship between biomass production and nitrogen fixation under drought stress conditions in peanut genotypes with different levels of drought resistance. *J. Agron. Crop Sci.* 194, 15-25.
- Puangbut, D., Jogloy, S., Vorasoot, N., Akkasaeng, C., Patanothai, A., 2011. Association of transpiration efficiency with N₂ fixation of peanut under early season drought. *Int. J. Plant Prod.* 5, 381-394.
- Reddy, T.Y., Reddy, V.R., Anbumozhi, V., 2003. Physiological responses of peanut (*Arachis hypogaea* L.) to drought stress and its amelioration: a critical review. *Plant Growth Regul.* 41, 75-88.

- Rucker, K.S., Kvien, C.K., Holbrook, C.C., Hook, J.E., 1995. Identification of peanut genotypes with improved drought avoidance traits. *Peanut Sci.* 22, 14-18.
- Schuman, G.E., Stanley, M.A., Knudson, D., 1973. Automated total nitrogen analysis of soil and plant samples. *Soil Sci. Soc. Am. Proc.* 37, 480-481.
- Serraj, R., Sinclair, T.R., Purcell, L.C., 1999. Symbiotic N₂ fixation response to drought. *J. Exp. Bot.* 50, 143-155.
- Sinclair, T.R., Leilah, A.A., Schreffler, A.K., 1995. Peanut nitrogen fixation (C₂H₂ reduction) response to soil dehydration. *Peanut Sci.* 16, 162-166.
- Singh, S., Russell, M.B., 1981. Water use by maize/pigeonpeasintercrop on a deep Vertisol. In: Proc. International workshop on pigeon peas vol. 1, ICRISAT Center Patancheru, Andhra Pradesh, India. Dec. 15-19, 1980. pp. 271-282.
- Songsri, P., Jogloy, S., Vorasoot, N., Akkasaeng, C., Patanothai, A., Holbrook, C.C., 2008. Root distribution of drought-resistant peanut genotypes in response to drought. *J. Agron. Crop Sci.* 194, 92-103.
- Songsri, P., Jogloy, S., Holbrook, C.C., Kesmala, T., Vorasoot, N., Akkasaeng, C., Patanothai, A., 2009. Association of root, specific leaf area and SPAD chlorophyll meter reading to water use efficiency of peanut under different available soil water. *Agric. Water Manage.* 96, 790-798.
- Thomas, Robertson, M.J., Fukai, S., Peoples, M.B., 2004. The effect of timing and severity of water deficit on growth, development, yield accumulation and nitrogen fixation of mungbean. *Field Crops Res.* 86, 67-80.
- Toomsan, B., Mc Donagh, J.E., Limpinuntana, V., Giller, K.E., 1995. Nitrogen fixation by groundnut and soybean and residual nitrogen benefits to rice in farmers' fields in Northeast Thailand. *Plant and Soil*, 175, 45-56.
- Venkateswarlu, B., Saharan, N., Maheswari, M., 1990. Nodulation and N₂ (C₂H₂) fixation in cowpea and groundnut during water stress and recovery. *Field Crops Res.* 25, 223-232.
- Vorasoot, N., Songsri, P., Akkasaeng, C., Jogloy, S., Patanothai, A., 2003. Effect of water stress on yield and agronomic characters of peanut (*Arachis hypogaea* L.). *Songklanakarin J. Sci. Technol.* 25, 283-288.
- Vorasoot, N., Akkasaeng, C., Songsri, P., Jogloy, S., Patanothai, A., 2004. Effect of available soil water on leaf development and dry matter partitioning in 4 cultivars of peanut (*Arachis hypogaea* L.). *Songklanakarin J. Sci. Technol.* 26, 787-794.
- Wright, G.C., Hubick, K.T., Farquhar, G.D., 1991. Physiological analysis of peanut cultivar response to timing and duration of drought stress. *Aust. J. Agric. Res.* 42, 453-470.
- Wright, G.C., Nageswara Rao, R.C., 1994. Groundnut water relations. In: J. Smartt, ed. *The Groundnut Crop*, Chapman & Hall, London, pp. 28-325.