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Effects of Drought on Characters Related to Nitrogen Fixation in Peanut

[1]S. Pimratch, [1]S. Jogloy, [1]N. Vorasoot, [1]B. Toomsan, [1]T. Kesmala, [1]A. Patanothai and [2]C.C. Holbrook
[1]Department of Plant Science and Agricultural Resources,
Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand
[2]Crop Genetics and Breeding Research Unit, USDA/ARS,
Coastal Plain Experiment Station, Tifton, Georgia 31793, USA

Abstract: Twelve peanut genotypes were tested under three water regimes in two greenhouses to investigate the effects of drought on biomass production and N_2 fixation. Drought reduced biomass production from 36.5 to 56.0% and reduced nitrogen fixation from 26.8 to 68.8%. Most genotypes with high biomass production under Field Capacity (FC) had high reduction in biomass production under drought conditions, but fewer genotypes with high N_2 fixed under FC showed high reduction in N_2 fixed. Biomass production under FC in general gave more contribution to biomass production under drought conditions than did the reduction. N_2 fixed under FC and the reduction in N_2 fixed contributed similarly to N_2 fixed under drought conditions. Positive and significant correlations between N_2 fixed and biomass production were found at FC and 2/3 available soil water (AW), but the correlation was not significant at 1/3 AW. Tifton-8 was the best genotype for high N_2 fixed under FC and KK 60-3 was the best genotype for low reduction. Correlations between N_2 fixed and nodule dry weight and shoot dry weight were high and consistent across water regimes. This information is important for breeders to develop peanut cultivars with reasonably high nitrogen fixation under drought conditions.

Key words: Biomass production, drought stress, N_2 fixation

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 N_2 fixation (Peoples *et al*., 1992; Serraj *et al*., 1999; Hungria and Vargas, 2000; Giller, 2001; Tomas *et al*., 2004). Depletion of soil fertility is also a major problem of crop production in rain-fed areas where the use of legumes such as *Sesbania* sp., as green manures to replenish soil fertility is rarely practiced (McDonagh *et al*., 1995; Toomsan *et al*., 2000). Pulse legumes such as peanut may be a good choice for this situation as they give dual utilities for growers. Peanut provides both residual nitrogen from its stover for succeeding crops and marketable pods for immediate cash (Toomsan *et al*., 1995). Although, access to irrigation can eliminate drought problem, it is limited for most peanut production areas and the most promising strategy to alleviate the problem is to use drought resistant varieties. However, peanut growers are reluctant to accept the drought resistant varieties if they do not give reasonable good yield under drought stress conditions.

Dry matter partitioning is very important in determination of crop yield (Bell *et al*., 1994). Total biomass has been used as a selection criterion for assessing drought resistance in peanut (Nageswara Rao *et al*., 1992). The drought resistant lines as identified by high biomass production were also more productive in yield than drought sensitive genotypes (Nageswara Rao *et al*., 1992; Nigam *et al*., 2003, 2005). Similar to biomass production, nitrogen fixation is important for growth and yield of leguminous crops especially in infertile soils. Drought greatly reduces nitrogen fixation, leading to low N accumulation, dry matter production and yield (Chapman and Muchow, 1985; Devries *et al*., 1989; De Silva *et al*., 1996).

Several authors so far have studied the effects of drought stress on N_2 fixation and its related traits in leguminous species (Hassan and Hall, 1987; Serraj *et al*., 1997, 1999; Serraj and Sinclair, 1996; Ramos *et al*., 1999) including peanut (Nambiar and Dart, 1983; Venkateswarlu *et al*., 1990; Sinclair *et al*., 1995; Pimratch *et al*., 2008a, b), but the studies were based on a small number of drought sensitive genotypes. It has been shown that shoot dry weight is correlated with nitrogen fixation and the use of shoot dry weight as a surrogate

Corresponding Author: Sanun Jogloy, Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen, 40002, Thailand Tel/Fax: +66-043- 364637

trait for selection of high nitrogen fixation is recommended under well-watered conditions (Nigam *et al*., 1985; Arrenddell *et al*., 1985; Pimratch *et al*., 2004). Most studies have focused on nitogenase activity as an indicator of N_2 fixation. Information on the reduction of N_2 fixed in shoot based on direct measurement and the relationships between N_2 fixed and its surrogate traits under mild and severe drought stresses is lacking.

In the previous study under field conditions, high N_2 fixation under drought stress could aid peanut genotypes in maintaining high yield under water limited conditions (Pimratch *et al*., 2008a). High potential under well-watered conditions contributed to high nitrogenase activity under mild drought but nitrogenase activity was dependent largely on low reduction under severe drought (Pimratch *et al*., 2008b). It is important to understand the factors (high potential of N_2 fixation under non-stress conditions or ability to maintain fixed N_2 under drought stress) contributing to high N_2 fixation under drought stress in order to develop appropriate breeding strategies for improving high N_2 fixation under drought stress. A better understanding of the relationship between N_2 fixation and shoot dry weight under water stress conditions should also have implications in breeding peanut for high N_2 fixation under drought stress.

The research at Khon Kaen University on drought stress in peanut has been conducted simultaneously both under greenhouse and field conditions since 2002 and all studies reported previously were conducted in the field. However, data in greenhouse study (December 2002 to November 2003) are also important but has not been reported elsewhere. The objective of this study was to determine in the greenhouse the effects of drought stress on biomass production, N_2 fixation, nodule number, nodule dry weight and shoot dry weight for peanut genotypes with different degrees of drought resistance.

MATERIALS AND METHODS

This research project was conducted under field and greenhouse conditions from December 2002 to April 2005 and the report herein was from the greenhouse experiment during December 2002 to November 2003.

Plant materials and experimental procedures: 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), one (Tifton-8) was a Virginia-type drought resistant line received from the United State Department of Agriculture (USDA), two (KK 60-3 and Tainan 9) were released cultivars commonly grown in Thailand and one was a non-nodulating line (Non-nod) included as reference plant in determining nitrogen fixation (McDonagh *et al*., 1993). The lines from ICRISAT were identified as drought resistant because they gave high total biomass and pod yield in screening tests under drought stress conditions (Nageswara Rao *et al*., 1992; Nigam *et al*., 2003, 2005). The KK 60-3 is a Virginia-type peanut cultivar with high N_2 fixation (Toomsan *et al*., 1995), but sensitive to drought for pod yield, while Tainan 9 is a Spanish-type peanut cultivar having low dry matter production (Vorasoot *et al*., 2003) and low N_2 fixation (McDonagh *et al*., 1993).

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) from December 2002 to May 2003 and was repeated from June 2003 to November 2003. A 3×12 factorial combination in a 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 (Yt; fine-loamy, siliceous, isohypothermic, Oxic Paleustults). The proportions of sand, silt and clay in the soil were 56.84, 24.79 and 18.37%, respectively. A sandy loam soil 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.

Pots having a diameter of 25 cm and height of 70 cm 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-isoindole-1,3(2H)-dione) at the rate of 5 g kg^{-1} 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. Three seeds were planted in each pot and plants were then thinned to 2 plants pot^{-1} 14 days after emergence (14 DAE). Phosphorus fertilizer as triple superphosphate at the rate of 12.12 g P pot^{-1} and potassium fertilizer as muriate of potash (KCl) at 15.26 g K pot^{-1} were applied 14 DAE.

Gypsum ($CaSO_4$) at the rate of 153.08 g pot^{-1} was applied 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, 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}.

The method of calculation for plant water use proposed by Songsri *et al.* (2008) 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 coefficient for peanut. The calculated amount of water was divided into four fractions. The first fraction was applied on the soil surface and the three factions 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 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, respectively. For each water level, soil moisture was controlled uniformly until harvest.

Data collection: Meteorological conditions and soil moisture: Rain fall, Relative Humidity (RH), evaporation (E0), maximum and minimum temperature and solar radiation were recorded daily from sowing until harvest by a weather station that was 50 and 750 m distant from greenhouse 1 (GH1) and greenhouse 2 (GH2), respectively. As the experiment was conducted in different seasons, the meteorological data were used for the calculation of crop water requirement for each season. Soil moisture and plant water status: Soil moisture was measured by the gravimetric method before planting and at harvest for both seasons. Briefly, 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 at constant moisture and the percent moisture could be 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 of the third leaf from the top of the main stem from one plant in each pot at 10-12 AM. RWC was measured following Gonzalez and Gonzalez-Vilar (2001), using the second leaf from the top of main stem from one plant in each pot. The same plant was also used for LWP measurement but the alternate plant in each pot was used in different times of evaluation. RWC was calculated as:

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

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

N_2-fixation and related traits: 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 dried at 75°C for 48 h 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 (Guebel *et al.*, 1991). 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.). Fixed nitrogen contents were calculated as: Fixed N_2 of each genotype = (Total N of each genotype)-(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 ^{15}N isotope dilution method in determining nitrogen fixation (McDonagh *et al.*, 1993; Bell *et al.*, 1994; Phoomthaisong *et al.*, 2003).

Percentages of reduction in biomass production and N_2 fixed from drought stress were used to evaluate the sensitivities of the genotypes to drought stress. Percentages of reduction in biomass production and N_2 fixed were calculated for each genotype as:

$$\text{Percentage of reduction of biomass} = \left[\frac{\text{1-(weight under stress)}}{\text{weight under non stress}}\right]\times 100$$

and

$$\text{Percentage of reduction of } N_2 \text{ fixed} = \left[\left(\frac{\text{1-}N_2\text{ fixed under stress}}{N_2\text{ fixed under non stress}}\right)\right]\times 100$$

Statistical analysis: Individual analysis of variance was performed for each character in each season (Gomez and

Gomez, 1984). Error variances for the two seasons were tested for homogeneity by Bartlett's test. Combined analyses of variance were done for those characters that error variances for the two seasons were homogeneous. Duncan's Multiple Range Test (DMRT) was used to compare means (Gomez and Gomez, 1984). The analysis of variance at this stage were done using MSTAT-C package.

Multiple-linear regression was used to determine the relative contribution of biomass production under non-stressed condition and reduction in biomass production under water stress condition to biomass production under each stress condition. The analysis was based on the following statistical model (Gomez and Gomez, 1984):

$$Y_i = \alpha + \beta_1 X_{1i} + \beta_2 X_{2i} + \delta_i$$

where, Y_i is biomass production under drought stress of genotype i, α is the Y intercept, X_{1i} and X_{2i} are biomass production under non-stress condition and reduction in biomass production under water stress condition of genotype i, respectively, β_1 and β_2 are regression coefficients for the independent variables X_1 and X_2 and δ_i is the associated deviation from regression.

The analysis was done by fitting the full model first and then determining the relative importance of the individual independent variables. A sequential fit was then performed by fitting the more important variable first. The relative contributions of the individual independent variables to biomass production under drought stress were determined from the percentages of regression sum of squares due to the respective independent variables to total sum of squares in the sequential fitted analysis.

Similarly, multiple regression analysis were done to determine the relative contributions of N_2 fixed under non-stress condition and reduction in N_2 fixed from drought stress to N_2 fixed under stress conditions. Simple correlation was used to determine the relationship between biomass production and fixed nitrogen, between N_2 fixed and traits related to N_2 fixed under drought stress conditions.

RESULTS

Meteorological conditions: The experiment in greenhouse 1 (GH1) was planted in December 2002 and finished in May 2003 and the experiment in greenhouse 2 (GH2) was planted in late June 2003 and finished in late November 2003. There were differences between the two greenhouses in temperature and relative humidity (Fig. 1a-e). In GH1, peanuts were exposed to low temperature in January and early February and the low temperature resulted in slow germination of seed and slow

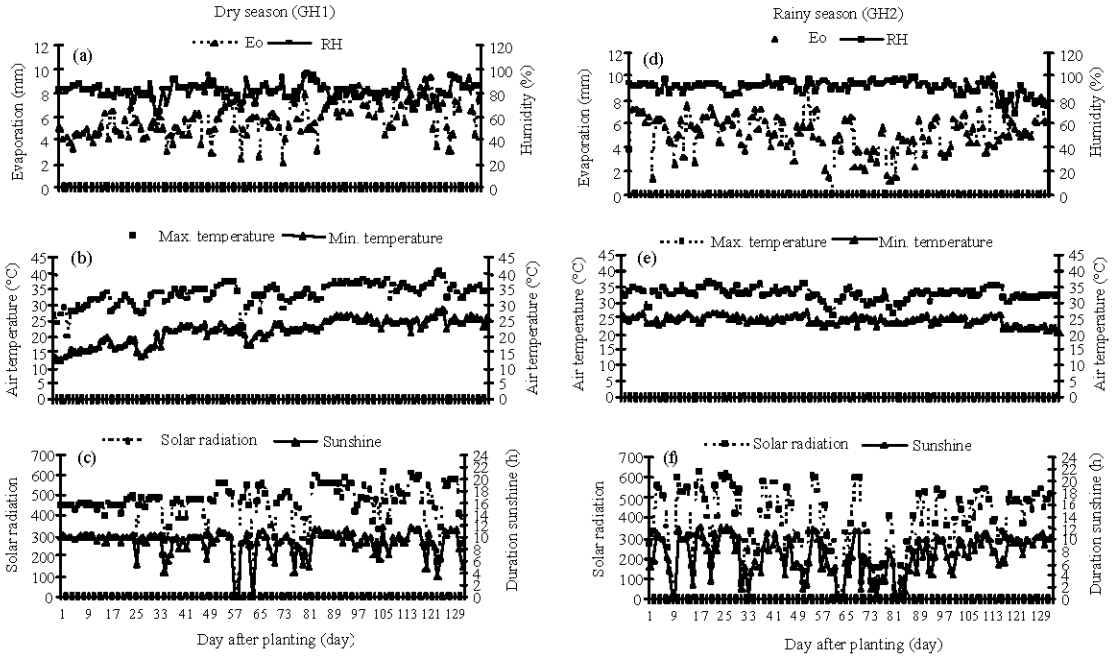

Fig. 1: (a-f) Evaporation (E0), humidity (RH), maximum temperature, minimum temperature, solar radiation and sunshine in dry season (a-c for greenhouse 1; GH1) and rainy season (d-f for greenhouse 2; GH2) in 2003

establishment of plants. The second greenhouse experiment was conducted during the wet season and relative humidity was higher than in GH1 (Fig. 1d). Solar radiation was higher in GH1 than in GH2 (Fig. 1c and f).

Soil moisture and leaf water status: Prior to initiation of experiments, soils were analyzed by pressure plate method to determine water holding capacity. 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 at 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 predetermined 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.

Leaf Water Potential (LWP) and Relative Water Content (RWC) were used as indicators for leaf water status. The LWP and RWC were significantly lower in the stressed treatments than the control (Fig. 2a-d). 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 the wilting in the 2/3 AW treatment in the afternoon, whereas plants in field capacity treatment were normal.

Combined analysis of variance for biomass production and N_2 fixation: Combined analysis of variance for the data from both experiments (Table 1) showed significant differences ($p<0.01$) between greenhouses, among water regimes and among peanut genotypes for both biomass production and N_2 fixed. Because season x genotype (S×G) interactions were significant ($p<0.01$) for both traits, the data for the two experiments are reported separately. S×W×G interactions were also significant for

Table 1: Mean square for the combined analyses of variance for biomass production and nitrogen fixation under greenhouse conditions in dry season and rainy season in 2003

		Mean square	
Source of variation	df	Biomass production	Nitrogen fixation
Season (S)	1	2193.0**	154966.8**
Reps. within season	10	11.8	1070.0
Water level (W)	2	5136.0**	183259.3**
SW	2	225.2**	41644.9**
Genotype (G)	10	33.2**	18854.7**
SG	10	14.3**	4239.1**
WG	20	16.1**	2362.0**
SWG	20	8.4**	1772.7*
Error	320	3.8	935.3
C.V. (%)	-	13.6	19.6

*, ** Significant at 95% and 99% probability levels, respectively

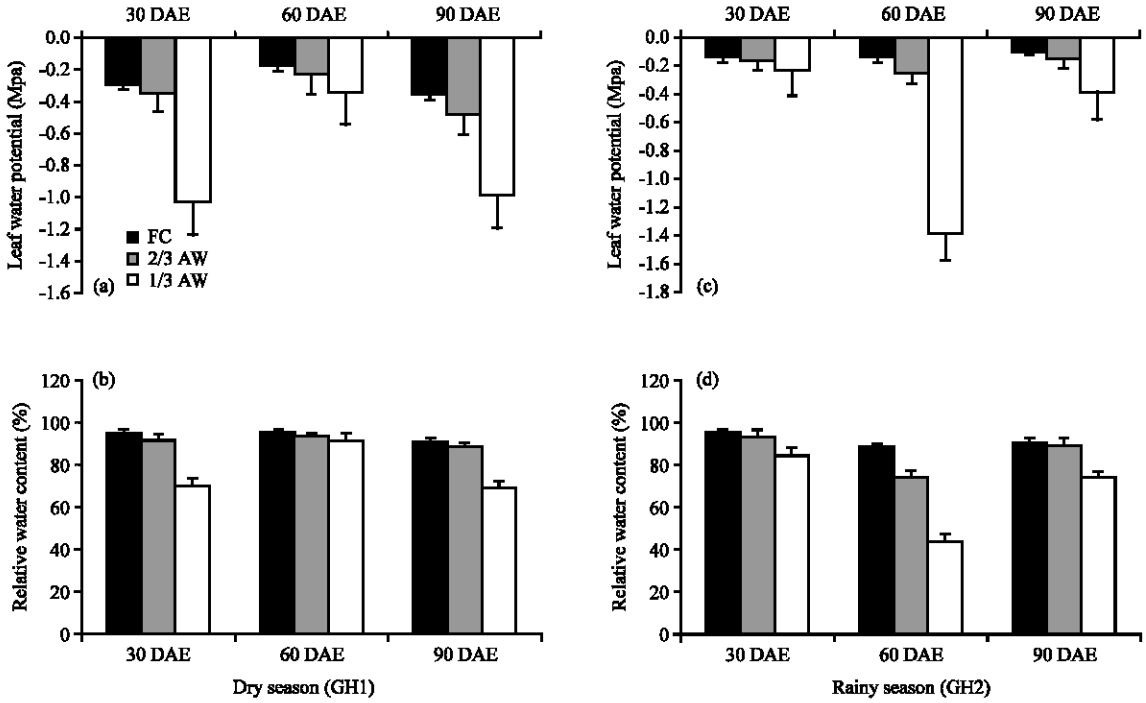

Fig. 2: Leaf water potential and relative water content at 30, 60 and 90 day after emergence (DAE) in dry season (a, b for greenhouse 1; GH1) and rainy season (c, d for greenhouse 2; GH2) in 2003

Table 2: Biomass production, the reductions in biomass production, N_2 fixation and the reductions in N_2 fixation of 11 peanut genotypes under different water regimes of greenhouse 1 and 2

	Biomass production (g plant^{-1})			Reduction in biomass production (%)		N_2 fixation(mg N plant^{-1})			Reduction in N_2 fixation (%)	
Genotypes	FC	2/3 AW	1/3 AW	2/3 AW	1/3 AW	FC	2/3 AW	1/3 AW	2/3 AW	1/3 AW
Greenhouse 1										
ICGV 98300	18.6a-c	11.7	7.4	37.2	59.9	81.8b	52.7cd	27.3c-e	35.6a-c	66.6a
ICGV 98303	15.1d	10.0	6.9	33.8	53.9	41.0cd	39.3de	33.7cd	4.1bcd	17.9bc
ICGV 98305	16.7cd	10.6	6.9	35.3	57.0	57.0b-d	40.7de	17.5e	28.6a-c	69.3a
ICGV 98308	16.4cd	10.9	7.6	33.2	53.0	59.0b-d	34.8e	24.5c-e	41.0ab	58.5a
ICGV 98324	16.4cd	11.3	8.2	31.5	49.6	74.0b-d	43.8de	36.7bc	40.8a-c	50.5ab
ICGV 98330	17.3b-d	9.9	8.5	42.9	50.9	92.8b	37.0de	29.0c-e	60.1a	68.8a
ICGV 98348	19.9ab	11.1	7.1	41.2	63.1	81.3b	66.7c	19.7de	18.0b-d	75.8a
ICGV 98353	15.6cd	10.0	7.6	35.2	51.2	39.8d	37.3de	21.8de	6.3cd	45.2ab
Tainan 9	16.3cd	10.2	8.4	37.1	47.8	78.3bc	51.0cde	51.0b	34.9a-c	34.9ab
KK 60-3	17.4b-d	11.1	7.3	32.4	55.0	89.0b	85.7b	73.0a	3.7d	18.0c
Tifton -8	21.5a	12.3	7.9	42.2	63.2	170.2a	134.0a	73.3a	21.3b-d	56.9a
Mean	17.4	10.8	7.6	36.5	55.0	78.6	56.6	37.0	26.8	51.1
Greenhouse 2										
ICGV 98300	26.6b-d	14.0	10.2cd	46.8a-c	60.8ab	163.0b-d	67.2cd	50.8b-e	58.8a	68.8
ICGV 98303	27.6bc	13.9	11.5ab	49.3a	58.0a-c	220.0a	79.3c	61.8bc	63.9a	71.9
ICGV 98305	23.1e-g	14.2	10.8b-d	38.5c	53.0cd	151.3c-e	65.3cd	69.3ab	56.8a-c	54.2
ICGV 98308	24.8c-f	13.4	10.1cd	45.2a-c	59.0a-c	147.3c-e	60.8cd	43.3c-e	58.7ab	70.6
ICGV 98324	22.1fg	13.2	11.5ab	39.5bc	46.8de	130.2de	57.2d	55.8b-e	56.1ab	57.1
ICGV 98330	26.0b-e	13.4	10.7b-d	48.2a-c	58.3a-c	198.0a-c	74.7cd	73.7ab	62.3ab	62.8
ICGV 98348	22.3fg	13.0	10.2cd	41.7a-c	54.4bc	155.5cd	67.0cd	57.7b-d	56.9a	62.9
ICGV 98353	23.6d-f	14.0	9.7d	39.9a-c	58.3a-c	140.2de	65.0cd	33.2e	53.6a-c	76.3
Tainan 9	20.4g	14.5	11.3a-c	27.7d	44.7e	102.2e	63.7cd	35.3de	37.7bc	65.4
KK 60-3	29.0ab	14.8	10.6b-d	48.7ab	62.9a	133.5de	116.2b	58.5b-d	13.0d	56.2
Tifton -8	30.8a	16.1	12.2a	47.6a-c	60.5ab	209.3ab	155.3a	87.7a	25.8cd	58.1
Mean	25.12	14.04	10.80	43.0	56.0	159.1	79.2	57.0	49.4	68.8

Different letters in each column show significant at 95% level of probability; FC: Field capacity, AW: Available soil water

biomass production ($p<0.01$) and N_2 fixed ($p<0.05$) and more extensive evaluation in more seasons will be necessary to more fully understand these interactions.

Greenhouse 1

Effects of water stress on biomass production: Drought stress greatly reduced biomass production of peanut by 36.5 and 55.0% for 2/3 AW and 1/3 AW, respectively (Table 2). Biomass productions of peanut genotypes, ranged from 15.1 to 21.5, 9.9 to 12.3 and 6.9 to 8.5 g plant^{-1} for FC, 2/3 AW and 1/3 AW, respectively, being significantly different ($p<0.01$) for FC only. Tifton-8 had the highest biomass production followed by the genotypes, ICGV 98348 and ICGV 98300, respectively. Reductions in biomass productions ranged from 31.5 to 42.9 and 47.8 to 63.2% for 2/3 AW and 1/3 AW, respectively and there were no statistical differences among these genotypes.

Factors contributing to biomass production under drought stress: The factors promoting high biomass production under drought was focused on high potential and low reduction. Biomass production under FC accounted for 58.1% of total contribution to biomass production under 2/3 AW, whereas the reduction in biomass production gave a smaller proportion of 35.1%

Table 3: Contribution of potential of biomass production at field capacity and the reduction of biomass production to biomass production and contribution of potential of N_2 fixed at field capacity and reduction in N_2 fixed to N_2 fixed under drought stress of greenhouse 1 and 2

Parameters	Greenhouse 1	Greenhouse2
Biomass production at 2/3 AW		
Regression	93.2**	97.6**
Potential of biomass production at FC	58.1**	61.5**
Reduction in biomass production at 2/3 AW	35.1**	36.1**
Biomass production at 1/3 AW		
Regression	95.4**	96.9**
Potential of biomass production at FC	66.9**	83.3**
Reduction in biomass production at 1/3 AW	28.5**	8.6**
N_2 fixed at 2/3 AW		
Regression	98.8**	96.1**
Potential of N_2 fixation at FC	78.4**	40.2**
Reduction in N_2 fixation at 2/3 AW	20.4**	55.9**
N_2 fixed at 1/3 AW		
Regression	95.5**	98.3**
Potential of N_2 fixation at FC	47.2**	50.8**
Reduction in N_2 fixation at 1/3 AW	48.3**	47.5**

*** Significant at 95 and 99% probability levels, respectively, FC: Field Capacity, AW: Available soil water

(Table 3). Under 1/3 AW, the contribution of biomass production under FC was still higher than that of the reduction, accounting for 66.9 and 28.5%, respectively.

The correlation coefficients between biomass production under FC conditions and the reduction in biomass were statistically significant at 2/3 AW ($r = 0.68^*$) and 1/3 AW ($r = 0.83^{**}$).

Effects of water stress on N_2 fixation: Similar to the results for biomass production, drought stress greatly reduced N_2 fixed by 26.8 and 51.1% under 2/3 AW and 1/3 AW, respectively (Table 2). Genotypes were significantly different ($p<0.01$) for N_2 fixed for all water levels, ranging from 39.8 to 170.2, 34.8 to 134.0 and 17.5 to 73.3 mg N/plant for FC, 2/3 AW and 1/3 AW, respectively. The differences in the reduction in N_2 fixation for both stress levels were also significant ($p<0.01$), ranging from 3.7 to 60.1 and 17.9 to 69.3% for 2/3 AW and 1/3 AW, respectively.

Interest has been focused on the genotypes that performed well under drought stress conditions to understand how these genotypes responded to different levels of available soil water. The variation in N_2 fixed under FC was not high enough to make clear differences among peanut genotypes. However, Tifton-8, KK 60-3, ICGV 98300, ICGV 98330 and ICGV 98348 were classified as genotypes with high N_2 fixation (Table 2). Tifton-8 was the most interesting because it showed high N_2 fixed at all water levels. Although, KK 60-3 were not as good as Tifton-8 under FC, it showed high N_2 fixed at both 2/3 AW and 1/3 AW. In contrast, ICGV 98300, ICGV 98330 and ICGV 98348 performed similarly to KK 60-3 under FC, but they had low N_2 fixed at both 2/3 AW and 1/3 AW.

Tifton-8 had relatively low reduction in N_2 fixed at 2/3 AW, but the reduction was quite high at 1/3 AW. KK 60-3 showed consistently low reduction at both stress levels. ICGV 98300 and ICGV 98330 had high reduction at both stress levels, whereas ICGV 98348 had low reduction at 2/3 AW, but it had high reduction at 1/3 AW.

Factors contributing to N_2 fixation under drought stress: The contributions of N_2 fixed under FC to N_2 fixed at 2/3 AW was greater than that of the reduction in N_2 fixed, accounting for 78.4 and 20.4%, respectively (Table 3). At 1/3 AW, however, the contributions of N_2 fixed under FC and the reduction were similar with the contributions of 47.2 and 48.3%, respectively.

The correlation coefficients between N_2 fixed under FC conditions and the reduction in N_2 fixed were not statistically significant at 2/3 AW ($r = 0.16$) and 1/3 AW ($r = 0.21$).

KK 60-3 had high N_2 fixed at 2/3 AW due to its high N_2 fixed under well-watered conditions and also due to its low reduction in N_2 fixed, whereas Tifton-8 had high N_2 fixed due largely to its high N_2 fixed under non-stress conditions (Table 2). In contrast to KK 60-3 and Tifton-8, ICGV 98303 and ICGV 98353 had low N_2 fixed because of their low N_2 fixed under FC conditions.

At 1/3 AW, KK 60-3 performed the best for N_2 fixed and its response was similarly to that at 2/3 AW in which the contributions of both factors were relatively high, whereas the high N_2 fixed of Tifton-8 was still dependent largely on its high N_2 fixed under well-watered conditions only. In contrast to KK 60-3 and Tifton-8, ICGV 98303 had low N_2 fixed because of low N_2 fixed under FC.

Table 4: Correlation between N_2 fixation and traits related to N_2 fixation under different water regimes of greenhouse 1 and 2

	N_2 fixation		
Parameters	FC	2/3 AW	1/3 AW
Greenhouse 1			
Nodule number	0.37	0.27	0.28
Nodule dry weight	0.86**	0.90**	0.88**
Shoot dry weight	0.93**	0.98**	0.81**
Biomass production	0.86**	0.72*	0.29
Greenhouse 2			
Nodule number	0.42	0.21	0.28
Nodule dry weight	0.80**	0.80**	0.33
Shoot dry weight	0.89**	0.89**	0.85**
Biomass production	0.68*	0.84**	0.58

*,** Significant at 95 and 99% probability levels, respectively, FC: Field capacity, AW: Available soil water

Relationship between N_2 fixation and its related traits: Correlations between N_2 fixed at harvest and its surrogate traits were studied to better understand the relationships between traits related to N_2 fixation. Shoot dry weight was consistently correlated with N_2 fixed across water regimes with the correlation coefficient ranging from 0.81** to 0.98** (Table 4). Nodule dry weight had good correlations with N_2 fixed ($r = 0.86^{**}$ to 0.90^{**}). Biomass production had good associations with N_2 fixed only at field capacity and 2/3 AW, whereas, the correlation coefficients between N_2 fixed and nodule number were not significant for any water regime.

Greenhouse 2

Effects of water stress on biomass production: Drought stress severely reduced biomass production by 43.0% and 56.0% for 2/3 AW and 1/3 AW, respectively (Table 2). Biomass productions of peanut genotypes at FC, 2/3 AW and 1/3 AW, ranged from 20.4 to 30.8, 13.0 to 16.1 and 9.7 to 12.2 g plant^{-1}, respectively. Significant genotypic differences were observed at FC and 1/3 AW only ($p<0.01$), but the differences in the reduction in biomass production were significant for both stress levels ($p<0.01$), ranging from 27.7 to 49.3 and 44.7 to 62.9% for 2/3 AW and 1/3 AW, respectively. Tifton-8 was the best genotype for high biomass production at both FC and 1/3 AW.

At 2/3 AW, Tainan 9 and ICGV 98305 were the best genotypes for lower reduction in biomass production, but the reductions of the others were rather high. At 1/3 AW, Tainan 9 was also the lowest genotype followed by ICGV 98324 and ICGV 98305, respectively.

Factors contributing to biomass production under drought stress: Biomass production under FC accounted for 61.5% of the total contribution to biomass production at 2/3 AW, whereas the reduction in biomass production accounted for 36.1% (Table 3). At 1/3 AW, however, biomass production under FC contributed by 83.3% compared to 8.6% for the reduction in biomass production.

The correlation coefficients between biomass production under FC conditions and the reduction in biomass were statistically significant at 2/3 AW ($r = 0.84^{**}$) and 1/3 AW ($r = 0.83^{**}$).

High biomass production of Tifton-8 under both 2/3 AW and 1/3 AW was dependent largely on high biomass production at FC, whereas, at 1/3 AW, high biomass productions of Tainan 9 and ICGV 98324 depended largely on low reduction in biomass (Table 2).

Effects of water stress on N_2 fixation: Similar to the results observed for biomass production, drought stress also reduced N_2 fixed by 49.4 and 68.8% for 2/3 AW and 1/3 AW, respectively (Table 2). N_2 fixed, ranged from 102.2 to 220.0, 67.0 to 155.3 and 33.2 to 87.7 mg N plant^{-1} for FC, 2/3 AW and 1/3 AW, respectively and significant genotypic differences were observed for all water levels ($p<0.01$). The differences in the reduction in N_2 fixation, ranged from 13.0 to 63.9 and 54.2 to 76.3% for 2/3 AW and 1/3 AW, respectively, but significant ($p<0.01$) were only observed at 2/3 AW.

ICGV 98303, Tifton-8, ICGV 98330 and ICGV 98300 had higher N_2 fixed under FC than the other genotypes. Tifton-8 was also the best genotype for N_2 fixed under both 2/3 AW and 1/3 AW, whereas KK 60-3, showed low N_2 fixed under FC, but had high N_2 fixed at 2/3 and relatively high at 1/3. ICGV 98330 and ICGV 98303 had high N_2 fixed under FC and 1/3 AW, but were not high at 2/3. Their performances at 1/3 AW were comparable to that of ICGV 98305, which showed low N_2 fixed both under FC and 2/3 AW.

Tifton-8 having consistently high N_2 fixed under all water levels showed the lowest reduction in N_2 fixed at 2/3 AW and was similar to KK 60-3 that showed high N_2 fixed at 2/3 but low under FC and 1/3 AW. At 1/3 AW, all genotypes were similar for the reduction in N_2 fixed. However, ICGV 98305, KK 60-3 and ICGV 98324 had relatively low reduction in N_2 fixed, although they had relatively low N_2 fixed under FC. ICGV 98303 had high N_2 fixed under FC, in contrast, this genotype showed relatively high reduction in N_2 fixed at both 2/3 AW and 1/3 AW.

Factors contributing to N_2 fixation under drought stress: The contribution of N_2 fixed under FC to N_2 fixed at 2/3 AW was 40.2% and lower than 55.9% contribution of the reduction in N_2 fixed (Table 3). The contributions of N_2 fixed under FC and the reduction in N_2 fixed to N_2 fixed at 1/3 AW were similar, accounting for 50.8 and 47.5%, respectively.

The correlation coefficients between N_2 fixed under FC conditions and the reduction in N_2 fixed were not statistically significant at 2/3 AW ($r = 0.23$) and 1/3 AW ($r = 0.09$).

These peanut genotypes exhibited different mechanisms for obtaining high N_2 fixation under different water stress levels. At 2/3 AW, both high potential (performed well under FC) and low reduction play a significant role in high N_2 fixed. The high N_2 fixed of Tifton-8 at 2/3 AW was clearly due to high N_2 fixed under well-watered conditions (Table 2). The KK 60-3 did not performed well for N_2 fixed under FC and thus it seemed to be dependent solely on its low reduction in N_2 fixed at 2/3 AW. ICGV 98303 and ICGV 98330 did not obtain high N_2 fixed under 2/3 AW although they had high N_2 fixed under FC conditions because they had high reduction, whereas ICGV 98324 had low N_2 fixed under water stress because of its low N_2 fixed under FC conditions and high reductions. Although, the reduction contributed a large proportion to N_2 fixed at 1/3 AW, peanut genotypes were not different and thus high potential played the most important role in obtaining high N_2 fixed at 1/3 AW. The genotypes with high N_2 fixed at 1/3 AW were Tifton-8 and ICGV 98330.

Relationship between N_2 fixation and related traits: The relationships between N_2 fixed and its surrogate traits in general were rather well associated and followed the similar patterns of those in greenhouse 1 (Table 4). Close associations were observed between N_2 fixed and shoot dry weight for all water regimes ($r = 0.85^{**}$ to 0.89^{**}). Most correlation coefficients between N_2 fixed and nodule dry weight and between N_2 fixed and total dry weight were significant ($r = 0.68^{*}$ to 0.84^{**}) except for the correlations between N_2 fixed and nodule dry weight and between N_2 fixed and total dry weight at 1/3 AW. Similar to what was observed in the first experiment, the correlation coefficients between N_2 fixed and nodule number were not significant for any water regimes.

DISCUSSION

Biomass production and nitrogen fixation in greenhouse 2 were higher than in greenhouse 1. This might be due to the fact that environmental conditions in greenhouse 2 favored the growth and development of peanut more than in greenhouse 1. Greenhouse 1 was carried out in the dry season and excessively low

temperature in January delayed early growth. In general, for both experiments biomass production and nitrogen fixation under FC were higher than under drought stress conditions and biomass production and nitrogen fixation under mild drought stress were higher than under severe drought stress. The results showed 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.

The differences in biomass production and nitrogen fixation among peanut genotypes especially under well-watered conditions in the two greenhouses allowed us to identify the genotypes with high or low potential for these traits and our discussion is focused on how they responded to drought stress and obtain high biomass production and nitrogen fixation under drought stress. For biomass production, the differences among peanut genotypes under water stress occurred at 1/3 AW in greenhouse 2 only. However, for nitrogen fixation, the differences occurred at both drought stress levels in both greenhouses. The variation in nitrogen fixation in the tested materials was somewhat higher than the variation in biomass production especially under water stress conditions.

All interaction effects in the combined analysis of variance were significant especially for genotype x greenhouse interaction, meaning that genotypes responded differently in two greenhouses. Because of the significant interactions, the separate results of each greenhouse were reported. The results also suggested that more extensive evaluation under a wide range of environments is needed to obtain a better understanding of these interactions. Differential responses of crop genotypes to varying environmental conditions are common for crop yield and other quantitative traits (Cooper and DeLacy, 1994; Wallace *et al*., 1993; Jackson *et al*., 1996).

Drought stress reduced biomass production and nitrogen fixation at 1/3 AW more than at 2/3 AW. These results were not unexpected because drought stress at 1/3 AW was more severe than at 2/3 AW. The reductions in biomass production were similar between the two greenhouse experiments, but the reduction in nitrogen fixation in greenhouse 2 was slightly higher than in greenhouse 1. Nitrogen fixation in peanut seemed to be more sensitive to environmental factors than biomass production. The higher reduction in N_2 fixed in greenhouse 2 than in greenhouse 1 might be caused by synergistic effect of water stress and high temperature in the greenhouse which was under closed environment with poor ventilation. The temperature in greenhouse 2 was 2°C higher than ambient environment, whereas the experiment in greenhouse 1 was carried out under open ventilation.

Although, there were several peanut genotypes that performed well for biomass production and nitrogen fixation under well-watered conditions, it is interesting to note that only Tifton-8 showed consistently high biomass production and high nitrogen fixation across water regimes and greenhouses. Other genotypes showed variable results for both biomass production and nitrogen fixation due to genotype by environment interactions.

High biomass production under well-watered conditions gave more contribution to high biomass production under drought stress than did the low reduction in biomass production. Similar pattern was also found for nitrogen fixation. The contribution of high nitrogen fixation under well-watered conditions was generally higher than did the reduction in nitrogen fixation under mild drought stress (2/3 AW). However, the contributions of high potential and low reduction became similar under severe drought stress (1/3 AW). The results supported previous findings under field conditions (Pimratch *et al*., 2008a).

High correlation between biomass production under FC and the reduction in biomass suggested that the genotypes with high potential also had high reduction in biomass production under drought stress conditions and thus transfer of these characters through conventional breeding may be difficult. The situation was different for nitrogen fixation in which the correlation between N_2 fixed under FC and the reduction in N_2 fixed were not associated for both greenhouses. This result suggests the possibility for combining these characters through conventional breeding.

Based on present results, Tifton-8 was the best genotype for high nitrogen fixation under FC and KK 60-3 was the best genotype for low reduction. They showed consistent performance across greenhouses and should be useful as germplasm sources for future crossing programs. Pimratch *et al.* (2008b) reported that Tifton-8 and KK 60-3 were the best genotypes for high nitrogenase activity under well-watered and mild drought stress conditions.

Variations in environmental factors can hinder progress in breeding for high nitrogen fixation in peanut when selection schemes are based on high biomass production or high nitrogen fixation. This is due to the complex nature of the inheritance of traits that are controlled by multiple genes (Scott *et al*., 2003). Arrendell *et al.* (1988) were not able to obtain genetic gains for high nitrogen fixation from selection in segregating peanut population due to high variation of

environments and large year effects. Because of the difficulty of selecting for nitrogen fixation, it may be useful to select for other characters with simple inheritance as surrogate traits for nitrogen fixation.

Shoot dry weight under drought stress may be useful selection criteria for high nitrogen fixation. Under normal moisture conditions and abundant soil nitrogen, nitrogen from soil plays a more important role in shoot accumulation than nitrogen derived from air (Marschner, 1995). However, under water-stressed conditions fixed nitrogen plays a more important role compared to that under non-stressed conditions. This means that peanut genotypes with high nitrogen fixation under drought stress tend to have higher shoot dry weight than genotypes with low nitrogen fixation.

Nodules obviously play an important role in nitrogen fixation because they are the plant part in which nitrogen is fixed. Present results showed consistently high associations between N_2 fixed and nodule dry weight, but there was no correlation between N_2 fixed and nodule number. These results indicated that large nodule size was more important than large number of nodules for high nitrogen fixation. Similarly, Rossum *et al.* (1993) observed that at low nodule number per plant the nodule size increased to generate sufficient nitrogen fixing tissue. This suggested that even at low number of nodules the nodules size can effectively fix sufficient amount of nitrogen to support plant growth and development.

Although, nodule dry weight and shoot dry weight might be useful, they are resource-intensive, requiring more time, labor and effort to measure. In early generations, shoot dry weight is considered to be the most suitable. However, the heritability of the traits and high G x E interaction must be considered.

CONCLUSION

Biomass production and N_2 fixation decreased with increasing levels of drought stress. Most genotypes with high biomass production under FC had high reduction in biomass production under drought conditions, but fewer genotypes with high N_2 fixed under FC showed high reduction in N_2 fixed. High biomass production under both 2/3 AW and 1/3 AW was due largely to high potential biomass production under FC and, to a lesser extent, the ability to maintain high biomass production under drought stress conditions. High N_2 fixation under both 2/3 AW and 1/3 AW was related to high potential N_2 fixation under FC and to a low rate of reduction in N_2 fixation in response to stress. Tifton-8 was the best genotype for high N_2 fixed under FC and KK 60-3 was the best genotype for low reduction under drought stress. Positive and significant correlations between N_2 fixed and biomass production were found at FC and 2/3 AW, but the correlation was not significant at 1/3 AW. Correlations between N_2 fixed and nodule dry weight and shoot dry weight were high and consistent across water regimes.

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