Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

Asian Journal of Plant Sciences 7 (4): 334-342, 2008
ISSN 1682-3974

Effect of Drought Stress on Traits Related to N_2 Fixation in Eleven Peanut (*Arachis hypogaea* L.) Genotypes Differing in Degrees of Resistance to Drought

[1]Sumran Pimratch, [1]Sanun Jogloy, [1]Nimitr Vorasoot, [1]Banyong Toomsan,
[1]Thawan Kesmala, [1]Aran Patanothai and [2]C. Corley 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: The aim of this study was to determine the effects of drought stress on nitrogenase activity, nodule number and nodule dry weight of eleven peanut (*Arachis hypogaea* L.) genotypes with different degrees of drought resistance. The relative values of these nitrogen fixation traits were evaluated under well watered and water-stressed conditions. Eleven peanut genotypes were tested in a split-plot design with four replications under field conditions in 2003/2004 and 2004/2005 in Northeast Thailand. Main-plot treatments were three water regimes [Field Capacity (FC), 2/3 Available soil Water (AW) and 1/3 AW] and sub-plot treatments were 11 peanut lines. Data were collected on nodule number, nodule dry weight and nitrogenase activity (acetylene reduction assay) at 30, 60 and 90 Days After Emergence (DAE). Severe drought stress reduced nitrogenase activity, nodule number and nodule dry weight about two times greater than did mild drought stress, causing uniform performance of peanut genotypes for nitrogenase activity under severe drought conditions. However, differences among peanut genotypes in nodule traits were found at all water levels. Tifton-8 and KK 60-3 in general performed better than the drought resistant lines from ICRISAT for all traits. Nodule dry weight was closely related with nitrogenase activity under drought conditions. High nitrogenase activity under mild drought conditions was related to high nitrogenase activity under well watered conditions (potential) and to a low rate of reduction in nitrogenase activity in response to stress. The contribution of the potential was lower under more severe drought conditions. Selection for high nitrogenase activity as a surrogate trait to improve nitrogen fixation under drought conditions should be more effective under mild than severe stress.

Key words: Nitrogen fixation, nitrogenase activity, potential, reduction, water stress

INTRODUCTION

Drought is one of the major causes of reduced growth and yield components in field crops. In leguminous plants, drought also reduces nitrogen fixation and its related traits. This phenomenon has been observed in peanut (*Arachis hypogaea* L.) (Sinclair *et al*., 1995), soybean (*Glycine max* Merr.) (Serraj *et al*., 1997), cowpea (*Vigna unguiculata* (L.) Walp.) (Hassan and Hall, 1987), mungbean (*Vigna radiata* L.) (Thomas *et al*., 2004) and common bean (*Phaseolus vulgaris* L.) (Ramos *et al*., 1999). As peanut is largely grown under rain-fed areas, where drought stress is a recurring problem and access to irrigation in these areas is limited, utilization of drought resistant genotypes is a viable alternative to alleviate the problem. The use of drought resistant cultivars should be of great advantage to retain high nitrogen fixation and acceptable yield. Nitrogen from the peanut residues is also beneficial for following crops such as rice (Toomsan *et al*., 2000), cassava (Toomsan *et al*., 1993) and maize (Phoomthaisong *et al*., 2003).

The cultivars with higher yield under drought stress conditions should fix more nitrogen than the cultivars with lower performance as symbiotic nitrogen fixation is important for growth and yield of leguminous crops (Rossum *et al*., 1993; Serraj *et al*., 1999) especially in infertile soils. However, in dry bean under well-watered conditions leaf nitrogen was not well associated with yield (Güler and Özcelik, 2007). High yielding soybean under drought stress fixed more nitrogen than drought sensitive cultivars (Serraj and Sinclair, 1996; Patterson and Hudak, 1996). This suggested that maintaining high N_2 fixation under drought stress could be a means for a legume genotype to achieve high yield under water-limited conditions.

Corresponding Author: Dr. Sanun Jogloy, Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen, 40002, Thailand Tel: 66-43- 342949 Fax: 66-43- 364636

Peanut genotypes differ in the responses to drought stress for the yield (Wright *et al*., 1991; Rucker *et al*., 1995; Collino *et al*., 2000) and traits related to nitrogen fixation (Nambiar and Dart, 1983; Venkateswarlu *et al*., 1990). The variations in these characters are important for selection of superior genotypes. A better understanding on how peanut genotypes responses to the drought stress for traits related to nitrogen fixation is also important for breeding peanut for high nitrogen fixation under drought stress. Present study clearly demonstrated that high nitrogen fixation under drought stress depended largely on high potential under well-watered conditions and for lesser extent on low reduction under water stress (Pimratch *et al*., 2008). It has been well demonstrated in peanut that nitrogen fixation is closely related to nodule traits and nitrogenase activity and they have been used as surrogate traits for nitrogen fixation (Nigam *et al*., 1985; Arrendrell *et al*., 1985; Pimratch *et al*., 2004). Sinclair *et al*. (1995) found insensitivity of nitrogenase activity to soil drying in peanut. They also found variation in drought sensitivity within a limited number of varieties. However, the responses for these traits to water stress have not been well documented in the literature especially in peanut genotypes with different levels of drought resistance.

The objective of this study was to determine of the eleven peanut (*Arachis hypogaea* L.) genotypes with different degrees of drought resistance response and the effect of drought stress on nitrogenase activity, nodule number and nodule dry weight by evaluating the relative values of these traits under the well watered and water-stressed conditions.

MATERIALS AND METHODS

The experiment was conducted under field conditions at the Field Crop Research Station of Khon Kaen University, Thailand (16°28' N and 102°48' E, asl 200 m) during the November 2003-May 2004 and October 2004-April 2005. The experimental site was exactly at the same field for both seasons. The soil of the experimental site belongs to Yasothon series (Yt; fine-loamy, siliceous, isohypothermic, Oxic Paleustults) (Phoomthaisong *et al*., 2003), soil properties are shown in Table 1. Experimental design was a split-plot in a randomized complete block design with four replications in both years. Main-plot treatments were three soil moisture levels [Field Capacity (FC), 2/3 available soil water (2/3 AW) and 1/3 AW] and sub-plot treatments were 11 peanut lines. Used peanut genotypes are (ICGV 98300, ICGV 98303, ICGV 98305, ICGV 98308, ICGV 98324, ICGV 98330, ICGV 98348, ICGV 98353, Tifton-8, KK 60-3 and Tainan 9. The lines from ICRISAT (Begins with ICGV) were identified as drought resistant because they gave high total biomass and pod yield in screening tests under drought stress conditions (Nageswar Rao *et al*., 1994; Nigam *et al*., 2003, 2005). 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). Plot size was 5×6 m with 50 cm spacing of between rows and 20 cm between plants. There were 300 plants in each plot with 2 plants per hill. Land preparation was done by plowing the field three times. Lime at 625 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 applied as a single dose prior to planting, but nitrogen fertilizer was not applied. Amount of fertilizer was calculated for each plot; and the fertilizer was broadcasted thoroughly and incorporated into the soil. 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 cultivars (KK 60-3 and Tifton-8) were also treated with ethrel 48% at the rate of 2 ml L^{-1} water to break dormancy. Three to four seeds were planted per hill by hand. *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 after planting and then water was applied at Field Capacity (FC). 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. Seed emergence was observed at 7 days after planting.

Table 1: Physical and chemical properties of soils from the experimental site at the depths 0-15 and 15-30 cm

Soil properties	2003-2004		2004-2005	
	0-15 cm	15-30 cm	0-15 cm	15-30 cm
Physical properties				
Sand (%)	93.700	91.000	93.700	91.200
Silt (%)	3.600	7.000	3.600	6.800
Clay (%)	2.700	2.000	2.700	2.000
Chemical properties				
pH	6.340	6.270	6.230	6.050
Electrical conductivity (m mhos cm^{-1})	0.052	0.048	0.091	0.083
Organic matter (%)	0.585	0.486	0.848	0.714
Total N (%)	0.029	0.025	0.039	0.036
Available P (ppm)	29.660	24.340	62.030	46.300
Exchangeable K (ppm)	40.220	26.060	64.410	57.270
Exchangeable Ca (ppm)	370.250	333.750	543.450	557.450
Exchangeable Mg (ppm)	-	-	31.680	28.520

The seedlings were thinned to two plants per hill at 14 Days After Emergence (DAE).

Gypsum ($CaSO_4$) at the rate of 312 kg ha^{-1} was applied at 40 DAE. Carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-ylmethylcarbamate 3% granular) was used at the pod setting stage. Pests and diseases were controlled by carbosulfan [2-3-dihydro-2,2-dimethylbenzofuran-7-yl (dibutylaminothio) methyl-carbamate 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 supply water to the crop and soil water level was maintained uniformly at field capacity from planting to 14 Days After Emergence (DAE). Afterward, soil moisture for the stress treatments were allowed to gradually decline until reaching the predetermined levels of 2/3 AW and 1/3 AW, respectively, then were held more or less constant until harvest. Soil moisture contents at FC and Permanent Wilting Point (PWP) were determined at 10.5 and 4.3%, respectively, by pressure plate method. Soil moisture contents for 2/3 AW and 1/3 AW were the values between FC and PWP that were proportional to soil moisture at FC. 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 (1980), respectively.

Data collection on soil moisture and plant water status: Soil moistures were measured by gravimetric method at planting and harvest at the depth of 0-5, 25-30 and 55-60 cm and changes in soil moisture levels were also monitored at 7 day intervals using neutron probe method at the depth of 30, 60 and 90 cm in dry seasons 2003/2004. Similar soil practices were done for dry seasons 2004/2005 but additional soil samples were collected for gravimetric moisture determination at 0, 15, 30, 60, 90 DAE and harvest.

In each plot, Leaf Water Potential (LWP) and Relative Water Content (RWC) were also measured at 30, 60 and 90 DAE to evaluate plant water status. LWP was determined by a pressure bomb model 1003 S/N 2973 (PMS Pressure bomb) at 10-12 am, using the third leaf from the top of the main stem and one leaf for each plot. RWC was measured following Kramer (1980), using the second leaf from the top of the main stem and 5 leaves for each plot. RWC was calculated as:

RWC = [(Fresh weight−dry weight)/(Saturated weight−dry weight)]× 100

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

Data collection on traits related to N_2-fixation: Nitrogenase activity was evaluated at 30, 60 and 90 DAE by acetylene reduction assay (Venkateswarlu *et al*., 1989) and read on a gas chromatography reader model GC-8A (Shimadzu Inc.). Ten plants in each plot were sampled for nitrogenase activity determination, then samples were washed with tap water and nodules were removed from roots, counted, oven dried (at 80°C for 48 h) and weighed.

Percentages of the reduction in nitrogenase activity, the nodule number and the nodule dry weight from drought stress were used to evaluate the sensitivities of the genotypes to the drought stress. Percentages of the reduction in these traits were calculated for each genotype as follows:

Percentage of reduction in nitrogenase activity = [1-(Nitrogenase activity under stress/Nitrogenase activity under non stress)]×100

Percentage of reduction in nodule number = [1-(Nodule number under stress/Nodule number under non stress)]×100

and

Percentage of reduction in nodule dry weight = 1-(Nodule dry weight under stress/Nodule dry weight under non stress)]×100

Statistical analyses: Individual analysis of variance was performed for each character in each year. Error variances for the two years were tested for homogeneity by Bartlett's test (Gomez and Gomez, 1984). Combined analyses of variance were done for those characters that had homogeneous error variances for the two years. The analyses of variance at this stage were done using MSTAT-C package (Bricker, 1989).

Simple correlation was used to determine the relationship between nitrogenase activity, with nodule number and nodule dry weight and between nodule number and nodule dry weight.

Multiple-linear regression was used to determine the relative contribution of nitrogenase activity under non-stressed condition and reduction in nitrogenase activity under water stress condition to nitrogenase activity under

each stress condition. The analysis was based on the following statistical model (Gomez and Gomez, 1984):

$$Y_i = a + \beta_1 X_{1i} + \beta_2 X_{2i} + d_i$$

where, Y_i is nitrogenase activity under drought stress of genotype i, a is the Y intercept, X_{1i} and X_{2i} are nitrogenase activity under non-stress condition and reduction in nitrogenase activity 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 d_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 the nitrogenase activity 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.

RESULTS AND DISCUSSION

Soil moisture and leaf water status: As can be shown in Fig. 1, at soil surface (30 cm), soil moistures of the three water regimes were clearly different for the dry season 2004/05, but the soil moistures of 2/3 and 1/3 AW in the dry season 2003/04 were not different at 75 DAE because of a rainfall of 86 mm at 68 DAE. The effect of this rainfall was also observed at the depth of 60 cm, causing high moistures for the 2/3 and 1/3 AW treatments. At 60 cm the well-watered treatment was still higher than the stress treatments, however, there was no difference between the stress treatments. At soil depth of 90 cm, the soil moistures of all water treatments were not different. However, soil moisture levels for different water regimes determined by percentages of available moisture content at different depths of the soil profile (0-5, 25-30 and 55-60 cm) in the 2003/2004 and 2004/2005 experiments showed a reasonable control of the water-regime treatments (data not reported).

As shown in Fig. 2, LWP and RWC values of the three water regimes were close at 30 DAE. Clearer distinctions were observed at 60 DAE and the best separations were observed at 90 DAE, indicating differences in levels of stress. Observation also found visual wilting at 2/3 AW and more severe wilting at 1/3 AW in the afternoon. Soil moisture contents were allowed to reduce to 2/3 AW at 21 DAE and 1/3 AW at 28 DAE. At 30 DAE, drought stress levels were not clearly different especially between 2/3 AW and 1/3 AW as indicated by soil moistures and leaf water status. Thus the evaluation data at 30 DAE were used for observation only. Leaf water potential also showed clearer differences among stress levels (Fig. 2a, c) in comparison to relative water contents possibly because of the low discriminating power of this parameter (Fig. 2b, d). These results indicated that degrees of stress were acceptably controlled at the desired levels. The major difference

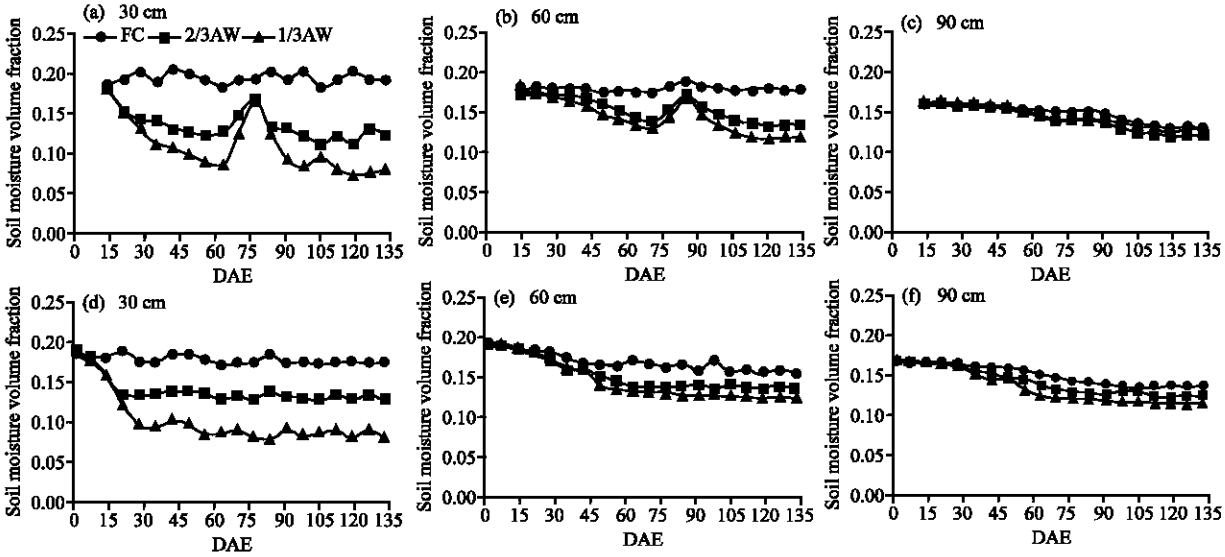

Fig. 1: Soil moisture volume fraction at the soil depth 30, 60 and 90 cm at 30, 60 and 90 Day After Emergence (DAE) of dry season 2003-2004 (a-c, respectively) and dry season 2004-2005 (d-f, respectively) estimated by Neutron probe

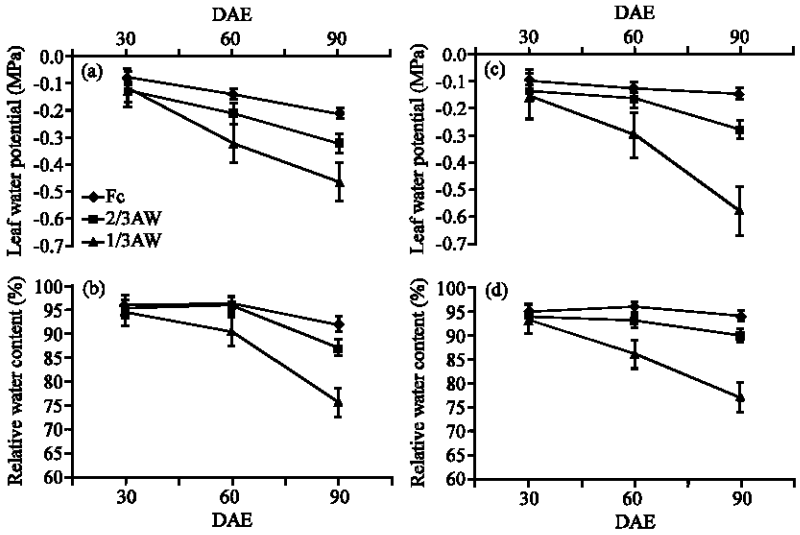

Fig. 2: Leaf water potential and relative water content at 30, 60 and 90 Day After Emergence (DAE) for the dry season 2003-2004 (a, b) and 2004-2005 (c, d)

Table 2: Nitrogenase activity Acetylene Reduction Assay (ARA), nodule number, nodule dry weight and the reductions in these traits of 11 peanut genotypes under different water regimes at 60 and 90 Day After Emergence (DAE)

	ARA					Nodule No.					Nodule dry weight				
	(μmole plant^{-1} h^{-1})			Reduction (%)		(Nodules plant^{-1})			Reduction (%)		(g plant^{-1})			Reduction (%)	
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	FC	2/3 AW	1/3 AW	2/3 AW	1/3 AW
60 DAE															
Tifton-8	15.7a	12.3a	7.5	21.4ab	52.1a	327cd	284abc	155b	12.2c	53.0cd	0.150cd	0.146b	0.080bc	1.6d	46.6abc
KK 60-3	15.6a	10.8ab	6.9	30.7a	55.7a	348b	307a	181a	11.7c	46.0d	0.208a	0.164a	0.110a	21.0abc	46.4abc
ICGV 98300	11.7b	9.6b	7.2	16.8abc	36.5bc	278f	230d	138bcd	16.2c	49.5d	0.174bc	0.132bcd	0.107a	18.5bcd	31.4c
ICGV 98348	11.0b	9.5b	6.8	12.6bc	37.3bc	296ef	184e	88g	34.1a	70.4a	0.192ab	0.110ef	0.084bc	38.1a	49.5ab
ICGV 98353	10.7b	10.5ab	6.9	4.0cd	34.9bc	286f	184e	100fg	30.3a	63.7b	0.150cd	0.080g	0.091abc	39.9a	34.1bc
ICGV 98324	11.3b	9.9b	7.5	12.0bc	32.6bc	373a	302a	148bc	20.7bc	61.1b	0.161cd	0.120de	0.073bc	24.2abc	52.3a
ICGV 98330	10.4b	10.2b	7.4	1.1d	28.2c	328cd	288ab	135cd	12.5c	59.2bc	0.143d	0.124cde	0.072c	8.3cd	46.7abc
ICGV 98308	11.2b	10.5ab	7.0	3.2cd	35.6bc	342bc	265c	129de	20.78bc	61.0b	0.159cd	0.119de	0.081bc	22.1abc	43.9abc
ICGV 98305	10.9b	9.9b	7.5	8.0bcd	30.4c	314de	267bc	113ef	15.34c	64.1ab	0.156cd	0.139bc	0.090abc	8.7cd	40.2abc
ICGV 98303	10.8b	10.2b	7.2	5.1cd	32.8bc	322d	264c	155b	16.8c	50.8cd	0.155cd	0.118de	0.095ab	18.6bcd	31.6c
Tainan 9	12.3ab	10.1b	7.1	16.7abc	41.0b	342bc	234d	110f	28.0ab	65.2ab	0.151cd	0.099f	0.074bc	29.9ab	45.8abc
Mean	12.0	10.4	7.2	12.0	37.9	295	255	132	19.9	58.5	0.164	0.123	0.087	21.0	42.6
90 DAE															
Tifton-8	12.6a	8.3a	4.9	34.4a	60.4a	345b	266a	165ab	19.7d	46.7e	0.227b	0.199a	0.118b	13.1e	50.4cde
KK 60-3	11.8ab	8.0ab	4.6	31.4ab	59.4a	379a	271a	175a	25.6cd	49.3e	0.237ab	0.183a	0.143a	22.1de	39.6e
ICGV 98300	10.3bc	7.5ab	4.3	27.4abc	57.5ab	285cd	157de	105e	44.6ab	62.0abc	0.242ab	0.143bc	0.085def	37.8bcd	61.9abc
ICGV 98348	9.5c	7.2b	4.4	24.1bcd	52.5abc	264d	130e	104e	48.9a	58.3bcd	0.229b	0.135bc	0.112bc	39.3bcd	46.8de
ICGV 98353	9.5c	7.4ab	4.4	22.6bcd	52.2abc	285cd	156de	83f	39.3abc	68.4a	0.183cd	0.073d	0.0578g	57.2a	65.8a
ICGV 98324	9.8c	7.7ab	4.3	21.6bcd	54.9abc	369ab	233b	122d	32.4bcd	62.0abc	0.201c	0.143bc	0.080ef	25.8cde	57.9a-d
ICGV 98330	9.2c	7.1b	4.7	23.7bcd	47.4c	367ab	220bc	132d	39.8abc	64.0ab	0.183cd	0.141bc	0.071fg	32.4bcd	62.5ab
ICGV 98308	9.3c	7.7ab	4.6	16.6d	48.5bc	291cd	174d	134cd	34.1bc	47.4e	0.204c	0.106cd	0.086def	47.6ab	57.3a-d
ICGV 98305	9.4c	7.8ab	4.6	16.7d	49.5bc	369ab	201c	150bc	43.9ab	54.1cde	0.252a	0.149b	0.098cd	40.6bc	60.5abc
ICGV 98303	9.6c	7.7ab	4.2	19.4cd	55.6abc	312c	172d	139cd	42.2ab	52.2de	0.182cd	0.136bc	0.092de	26.2cde	51.0b-e
Tainan 9	10.6bc	7.4ab	4.3	31.1ab	59.1a	271d	171d	92ef	34.7abc	63.9ab	0.162d	0.106cd	0.058g	33.8bcd	63.8a
Mean	10.1	7.6	4.5	24.4	54.3	322	196	127	36.8	57.1	0.209	0.138	0.091	34.2	56.1

Different letter(s) in each column show significant at 99% level of probability; FC: Field Capacity, AW: Available soil water, *,**Significant at 95 and 99% probability levels, respectively

between the two seasons was caused mainly by the rainfall that occurred at 68 DAE during the 2003/04 season.

Responses for nitrogenase activity, nodule number and nodule dry weight: Data for the two years were combined because the interaction effects for genotypes × year were relatively low for all characters compared to main genotype effects (data not reported). The genotypes were significantly different for most evaluations except for nitrogenase activity at 1/3 AW at 60 and 90 DAE (Table 2).

Drought stress reduced nitrogenase activity, nodule number and nodule dry weight, but the reductions in

these traits differed among traits and between levels of drought stress. The results indicated that levels of stress and times of evaluations had significant effects on the responses of peanut genotypes for these traits. For nitrogenase activity, the reductions at 60 DAE (12.0% for 2/3 AW and 37.9% for 1/3 AW) were much lower than those at 90 DAE (24.4% for 2/3 AW and 54.3% for 1/3 AW). As nitrogenase activity requires assimilates from aerial parts, the higher reduction in nitrogenase activity at 90 DAE could be due to high competition of developing pod. For nodule number, a large difference in the reduction was found at 2/3 AW (19.9% for 60 DAE and 36.8% for 90 DAE), but similar reductions at 60 and 90 DAE were found at 1/3 AW (58.5% for 60 DAE and 57.1% for 90 DAE). The difference in the reduction in nodule number between the two stress levels might be due to the difference in nodule establishment and formation as affected by drought stress at different levels. Under severe stress conditions, establishment of nodules was limited as indicated by the high reduction in nodule number at 60 DAE and the high reduction in nodule number lasted until 90 DAE. However, the establishment and formation of nodules in peanut are continuous processes and, under well-watered conditions, the most nodules and the most effective nodules per plant occur by 100 days after sowing (Wahhab and Bhuiya, 1984). For nodule dry weight, the reductions at 90 DAE (34.2% for 2/3 AW and 56.1% for 1/3 AW) were higher than those at 60 DAE (21.0% for 2/3 AW and 42.6% for 1/3 AW). However, nodule dry weights increased at 90 DAE for all water regimes, but the reduction pattern was similar to that of nitrogenase activity rather than nodule number. The results showed that nodule dry weight could develop even under stress conditions and therefore low numbers under stress conditions could be partially compensated by larger nodules.

The variation in nitrogenase activity was not high and significant differences among genotypes were found at FC and 2/3 AW only. This could be due to high reduction in nitrogenase activity under severe drought conditions. Tifton-8 and KK 60-3 were the best genotypes, showing consistently higher nitrogenase activity than other genotypes. The reductions in nitrogenase activity of Tifton-8 and KK 60-3 were also higher than those of the others.

The variation in nodule number was rather high compared to that of nitrogenase activity and significant differences among peanut genotypes were observed at all water regimes and both sampling dates. The genotypes performing well at FC did not necessarily have high reduction and vice versa. Interest has been focused on the genotypes with high nitrogenase activity under drought conditions to understand the mechanisms underlying the high performance. This is because most genotypes had been carefully selected for their resistance to drought in experiments in which pod yield and biomass production were the main criteria. The drought resistant lines from ICRISAT did not differ greatly for nitrogenase activity and nodule traits. Tifton-8, a drought resistant line from the USDA had a moderate number of nodules at FC, but relatively high numbers under drought conditions especially at 90 DAE. This might be due to low reductions at both stress levels. KK 60-3 had a high number of nodules at FC and it also performed well under drought conditions at both sampling dates. Low reduction in nodule numbers was also the cause.

Similar to nodule number, high variation was found for nodule dry weight and significant differences among genotypes were found for all water levels and sampling dates. KK 60-3 showed consistently high nodule dry weight across water levels and sampling dates. This was due to high performance at FC and relatively low reduction. Nodule dry weight for Tifton-8 was not as high as that of KK 60-3 at 60 DAE under well-watered conditions, but similar at 90 DAE. Under drought conditions, Tifton-8 has lower nodule dry weight than did KK 60-3 at 60 DAE, but its nodule number was similar to that of KK 60-3 at 90 DAE. Tifton-8 also had relatively low reduction in nodule dry weight in response to water stress.

Studies have reported a good association of N_2 fixed and the nitrogenase activity (Wynne *et al*., 1980; Phillips *et al*., 1989). The genotypes performing well for the nitrogenase activity under drought conditions could be due to two different mechanisms, high potential or low reduction, or both. High nodule number and nodule weight could also enhance the nitrogenase activity. In this case, Tifton-8 and KK 60-3 showed differential responses for nodule traits although the responses for nitrogenase activity were similar. KK 60-3 performed consistently well for the nitrogenase activity and nodule traits, whereas Tifton-8 had relatively lower nodule traits at 60 DAE and the development of nodules was slow at early growth (30 DAE, data not reported). Tifton-8 has been reported as drought resistant because of high root mass (Coffelt *et al*., 1985), while KK 60-3 has been reported to have high biomass production (Pimratch *et al*., 2008) but drought sensitive for pod yield (Vorasoot *et al*., 2003). KK 60-3 and Tifton-8 are both Virginia botanical type that have longer crop duration and growth development, while the other genotypes are all Spanish botanical types. Virginia genotypes in general have greater nitrogen fixation (Elkan *et al*., 1980; Nambiar and Dart, 1983; Phillips *et al*., 1989).

Table 3: Correlation between nitrogenase activity Acetylene Reduction Assay (ARA), with nodule number and nodule dry weight different water regimes

	Nitrogenase activity		
Parameters	FC	2/3 AW	1/3 AW
60 DAE			
Nodule number	0.28	0.37	0.30
Nodule dry weight	0.24	0.68*	0.63*
90 DAE			
Nodule number	0.39	0.35	-0.37
Nodule dry weight	0.28	0.65*	0.42
	Nodule dry weight		
Nodule number			
60 DAE	-0.03	0.72*	0.35
90 DAE	0.29	0.74**	0.77**

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

Table 4: Contribution of potential of nitrogenase activity at field capacity and reduction in nitrogenase activity fixed under drought stress at 60 and 90 Days After Emergence (DAE)

	Explained by regression (%)	
	60 DAE	90 DAE
At 2/3 AW		
Regression	96.20**	98.8**
Potential of nitrogenase activity at FC	55.10**	52.4**
Reduction in nitrogenase activity at 2/3 AW	41.10**	46.5**
At 1/3 AW		
Regression	94.60**	98.2**
Potential of nitrogenase activity at FC	0.10**	19.1**
Reduction in nitrogenase activity at 1/3 AW	94.50**	79.1**

**Significant at the 0.01 probability level, FC: Field Capacity, AW: Available soil Water

Table 3 shows relationships among nitrogenase activity, nodule number and nodule dry weight. Significant correlation coefficients were not found under FC, but significant correlation coefficients were found between the nodule dry weight and the nitrogenase activity at 2/3 AW at both sampling dates (r = 0.68* and 0.65* for 60 and 90 DAE, respectively) and at 1/3 AW at 60 DAE (r = 0.63*). The results indicated that nodule dry weigh was more important than nodule number for nitrogen fixation under drought conditions and also implied that nitrogen derived from the air is necessary for the crop as nitrogen uptake is limited by drought. The results were in agreement with several studies mostly conducted under well-watered conditions for the correlations of these traits (Nigam *et al*., 1985; Pimratch *et al*., 2004). However, the lack of correlations under well-watered conditions in our study could be due to the high nitrogen uptake that the roots can achieve under non-stressed conditions. It should be noted that at, 1/3 AW at 90 DAE, the correlation coefficient between nodule number and the nitrogenase activity was negative but the correlation coefficient between the nodule dry weight and the nitrogenase activity was positive though not significant. This also supported the importance of nodule dry weight to nitrogen fixation under stressed conditions. Nodule number and nodule dry weight were well associated under drought conditions especially at 90 DAE (r = 0.74** and 0.77** for 2/3 and 1/3 AW, respectively).

Nitrogenase activity under well-watered conditions and the amount of reduction under drought conditions have to be determined to formulate appropriate breeding strategies. Regression analysis showed that at 2/3 AW the joint contributions of nitrogenase activity under well-watered conditions and the amount of reduction under stress were high, accounting for 96.2 and 98.8% of total contributions at 60 and 90 DAE, respectively (Table 4). The reductions (41.1% for 60 DAE and 46.5% for 90 DAE) were slightly lower than those of nitrogenase activity under well-watered conditions (55.1% for 60 DAE and 52.4% for 90 DAE). The results indicated that both potential and reduction are equally important for the performance under mild drought. Under severe drought, the relative contributions of these factors were also high, with total contributions of 94.6 and 98.2% for 60 and 90 DAE (Table 4), respectively. However, the contributions of the reduction, accounting for 94.5 and 79.1% of total contributions at 60 and 90 DAE, respectively, were much higher than those of nitrogenase activity under well-watered conditions (0.10% for 60 DAE and 19.1% for 90 DAE). These results indicated that under severe drought the performance of peanut genotypes for nitrogenase activity was dependent largely on the reduction.

It is clear from the results that the contributions of potential and reduction in the nitrogenase activity were different between drought stress levels. The contribution of potential was high under mild drought and it was reduced with more severe stress. In contrast, the contribution of the reduction in the nitrogenase activity was increased with increasing drought stress. The results were in agreement with contributions of potential and the reduction for N_2 fixed in our parallel study (Pimracth *et al*., 2008). From a plant breeding point of view, selection for high nitrogenase activity as a surrogate trait to improve nitrogen fixation in peanut under drought conditions should be effective if the drought is not too severe.

In conclusion, drought stress reduced the nitrogenase activity, nodule number and nodule dry weight and the reductions for these traits at severe drought stress were about two times the reductions under mild drought stress. Drought stress at 1/3 AW was too severe, resulting in uniform performance of peanut genotypes for the nitrogenase activity. However, the differences among peanut genotypes in nodule number and nodule weight were significant even under severe drought conditions. Virginia peanut genotypes in general

performed better than did the drought resistant Spanish lines from ICRISAT for the nitrogenase activity, nodule number and nodule dry weight. The reductions in nitrogenase activity were higher in Virginia peanuts, but the reductions in nodule number were lower, whereas the reductions in nodule dry weight were rather similar and genotype-dependent. Potential of the nitrogenase activity contributed to the performance under mild drought conditions greater than the reduction in this trait, but the contribution of the potential was lower under severe drought conditions. Soil moisture levels affect the performance and relationships between nitrogen fixation traits and have important implications on breeding peanut for drought resistance.

ACKNOWLEDGMENTS

The authors are grateful for the financial support of the Royal Golden Jubilee Ph.D. Program (Grant No. PHD/0106/2547) and the Senior Research Scholar Project of Professor Dr. Aran Patanothai under the Thailand Research Fund and also supported in part by the Peanut Improvement Project of Khon Kaen University. Very thankful acknowledgments have been given to Dr. S.N. Nigam (ICRISAT, India) for the donation of peanut seed. The contribution of Dr. Viboon Pensuk (Udon Thani Rajabhat University) for his research facility and equipments are greatly acknowledged. Also, we thank the work of many people during the study.

REFERENCES

Arrendrell, S., J.C. Wynne, G.H. Elkan and T.G. Isleib, 1985. Variation for nitrogen fixation among progenies of a Virginia×Spanish peanut cross. Crop Sci., 25 (5): 865-869.

Bricker, A.A., 1989. MSTAT-C User's Guide. Michigan State University. East Lansing, MI.

Coffelt, T.A., R.O. Hammons, W.D. Branch, R.W. Mozingo, P.M. Phipps, J.C. Smith, R.E. Lynch, C.S. Kvien, D.L. Ketring, D.M. Porter and A.C. Mixon, 1985. Registration of Tifton-8 peanut germplasm. Crop Sci., 25 (1): 203.

Collino, D.J., J.L. Dardanelli, R. Sereno and R.W. Racca, 2000. Physiological responses of argentine peanut varieties to water stress. Water uptake and water use efficiency. Field Crops Res., 68 (2): 133-142.

Doorenbos, J. and W.O. Pruitt, 1992. Calculation of Crop Water Requirements. In: Crop Water Requirements. FAO Irrigation and Drainage Paper No. 24, Rome, Italy, pp: 1-65.

Elkan, G.H., J.C. Wynne, T.J. Schneeweis and T.G. Isleib, 1980. Nodulation and nitrogenase activity of peanuts inoculated with single strain isolates of *Rhizobium*. Peanut Sci., 7 (2): 95-97.

Gomez, K.A. and A.A. Gomez, 1984. Statistical Procedures for Agricultural Research. John Wiley and Sons, New York.

Güler, S. and H. Özcelik, 2007. Relationship between leaf chlorophyll and yield related characters of dry bean (*Phaseolus vulgaris* L.). Asian J. Plant Sci., 6 (4): 700-703.

Hassan, O.A.E. and A.E. Hall, 1987. Influences of early and late nitrogen fertilization on yield and nitrogen fixation of cowpea under well-watered and dry field conditions. Field Crops Res., 15 (3-4): 229-244.

Kramer, P.J., 1980. Drought, Stress and the Origin of Adaptation. In: Adaptation of Plant to Water and High Temperature Stress, Turner, N.C. and P.J. Kramer (Eds.). John Wiley and Sons, New York, pp: 7-20.

McDonagh, J.F., B. Toomsan, V. Limpinantana and K.E. Giller, 1993. Estimates of the residual nitrogen benefit of groundnut to maize in Northeast Thailand. Plant Soil, 154 (2): 267-277.

Nageswar Rao, R.C., L.J. Reddy, V.K. Mehan, S.N. Nigam and D. McDonald, 1994. Drought research on groundnut at ICRISAT. In: Proceedings of an International Workshop Groundnut a Global Perspective, ICRISAT, Patancheru, A.P., India, pp: 455.

Nambiar, P.T.C. and P.J. Dart, 1983. Factors influencing nitrogenase activity (acetylene reaction) by root nodules of groundnut, *Arachis hypogaea* L. Peanut Sci., 10 (1): 26-29.

Nigam, S.N., S.L. Dwivedi, P.T.C. Nambiar, R.W. Gibbons and P.J. Dart, 1985. Combining ability analysis of N_2–fixation and related traits in peanut. Peanut Sci., 12 (2): 55-57.

Nigam, S.N., M.S. Basu and A.W. Cruickshank, 2003. Hybridization and description of the trait-based and empirical selection programs. In: Breeding of Drought-Resistant Peanuts. Proceedings of a Collaborative Review Meeting Held at ICRISAT Centre, ICRISAT, Hyderabad, A.P., India. Canberra, ACIAR Proceedings No. 112, pp: 15-17.

Nigam, S.N., S. Chandra, K.R. Sridevi, M. Bhukta, A.G.S. Reddy, N.R. Rachaputi, G.C. Wright, P.V. Reddy, M.P. Deshmukh, R.K. Mathur, M.S. Basus, S. Vasundhara, P.V. Varman and A.K. Nagda, 2005. Efficiency of physiological trait-based and empirical selection approaches for drought tolerance in groundnut. Annu. Applied Biol., 146 (4): 433-439.

Patterson, P.R. and C.M. Hudak, 1996. Drought-avoidant soybean germplasm maintains nitrogen fixation capacity under water stress. Plant Soil, 186 (1): 39-43.

Phillips, T.D., J.C. Wynne, G.H. Elkan and T.J. Schneeweis, 1989. Field evaluations of peanut cultivar *Bradyrhizobium* specificities. Peanut Sci., 16 (1): 54-57.

Phoomthaisong, J., B. Toomsan, V. Limpinuntana, G. Cadisch and A. Patanothai, 2003. Attributes affecting residual benefits of N_2-fixing mungbean and groundnut cultivars. Biol. Fert. Soils, 39 (1): 16-24.

Pimratch, S., S. Jogloy, B. Toomsan, P. Jaisil, J. Sikhinarum, T. Kesmala and P. Patanothai, 2004. Evaluation of seven peanut genotypes for nitrogen fixation and agronomic traits. Songklanakarin J. Sci. Technol., 26 (3): 295-304.

Pimratch, S., S. Jogloy, N. Vorasoot, B. Toomsan, A. Patanothai and C.C. Holbrook, 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 (1): 15-25.

Ramos, M.L.G., A.J. Gordon, F.R. Minchin, J.I. Sprent and R. Parsons, 1999. Effect of water stress on nodule physiology and biochemistry of a drought tolerant cultivar of common bean (*Phaseolus vulgaris* L.). Annu. Bot., 83 (1): 57-63.

Rossum, D.V., A. Muyotcha, H.W.V. Verseveld, A.H. Stouthamer and F.C. Boogerd, 1993. Effects of *Bradyrhizobium* strain and host genotype, nodule dry weight and leaf area on groundnut (*Arachis hypogaea* L. sp. *fastigiata*) yield. Plant Soil, 154 (2): 279-288.

Rucker, K.S., C.K. Kvien, C.C. Holbrook and J.E. Hook, 1995. Identification of peanut genotypes with improved drought avoidance traits. Peanut Sci., 22 (1): 14-18.

Serraj, R. and T.R. Sinclair, 1996. Processes contributing to N_2-fixation insensitivity to drought in the soybean cultivar Jackson. Crop Sci., 36 (4): 961-968.

Serraj, R., S. Bona, L.C. Purcell and T.R. Sinclair, 1997. Nitrogen fixation response to water-deficits in field-grown 'Jackson' soybean. Field Crops Res., 52 (1-2): 109-116.

Serraj, R., T.R. Sinclair and L.C. Purcell, 1999. Symbiotic N_2 fixation response to drought. J. Exp. Bot., 50 (331): 143-155.

Sinclair, T.R., A.A. Leilah and A.K. Schreffler, 1995. Peanut nitrogen fixation (C_2H_2 reduction) response to soil dehydration. Peanut Sci., 16 (2): 162-166.

Singh, S. and M.B. Russell, 1980. Water use by maize/pigeonpea intercrop on a deep Vertisol. In: Proceedings of the International Workshop on Pigeonpeas ICRISAT Center Patancheru, India, 1: 271-282.

Thomas, M.J.R., S. Fukai and M.B. Peoples, 2004. The effect of timing and severity of water deficit on growth, development, yield accumulation and nitrogen fixation of mungbean. Field Crops Res., 86 (1): 67-80.

Toomsan, B., V. Limpinuntana, J. Homchan, P. Sripa, P. Paopuri, S. Manikan, J. Sanitchon, M. Srichantawong and S. Saranun, 1993. Nitrogen fixation of groundnut and its residual nitrogen benefits to succeeding cassava. In: Proceedings of the 11th Thailand National Groundnut Meeting, the Chansomthara Hotel, Ranong, 17-21 May 1993. Published by Department of Agricultural Extension, Ministry of Agriculture and Cooperatives, pp: 465-475.

Toomsan, B., J.F. McDonagh, V. Limpinuntana and K.E. Giller, 1995. Nitrogen fixation by groundnut and soybean and residual nitrogen benefits to rice in farmers' fields in Northeast Thailand. Plant Soil, 175 (1): 45-56.

Toomsan, B., G. Cadisch, M. Srichantawong, C. Tongsodsaeng, K.E. Giller and V. Limpinuntana, 2000. Biological N_2 fixation and residual N benefit of pre-rice leguminous crops and green manures. Neth. J. Agric. Sci., 48 (1): 19-29.

Venkateswarlu, B., M. Maheswari and N. Saharan, 1989. Effects of water deficit on N_2 (C_2H_2) fixation in cowpea and groundnut. Plant Soil, 114 (1): 69-74.

Venkateswarlu, B., N. Saharan and M. Maheswari, 1990. Nodulation and N_2 (C_2H_2) fixation in cowpea and groundnut during water stress and recovery. Field Crops Res., 25 (3-4): 223-232.

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

Wahhab, M.A. and Z.H. Bhuiya, 1984. Abundance and distribution of nodules in root system of groundnut. Bangladesh J. Agic. Res., 9 (1): 97-102.

Wright, G.C., K.T. Hubick and G.D. Farquhar, 1991. Physiological analysis of peanut cultivar response to timing and duration of drought stress. Aust. J. Agric. Res., 42 (3): 453-470.

Wynne, J.C., G.H. Elkan, C.M. Meisner, T.J. Schneeneis and J.M. Ligon, 1980. Greenhouse evaluation of strains of *Rhizobium* of peanuts. Agron. J., 72 (4): 645-649.