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BIOLOGICAL NITROGEN FIXATION OF PEANUT GENOTYPES WITH DIFFERENT LEVELS OF DROUGHT TOLERANCE UNDER MID-SEASON DROUGHT

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SUMMARY

The objective of this study was to investigate the responses of peanut genotypes to mid-season drought for nitrogen fixation traits and their correlations with agronomic traits and pod yield. The experiment was conducted at Khon Kaen University's Agronomy Farm, Khon Kaen Province, Thailand during dry season 2011/12. Five peanut genotypes with different levels of tolerance to mid-season drought and 2 soil moisture regimes (well-watered and drought stress during mid-season) were laid out in a split-plot design with 4 replications. Mid-season drought was initiated by stopping irrigation at 30 days after planting (DAP) and then re-watering at 60 DAP. The data were collected for SPAD Chlorophyll Meter Reading (SCMR) at 75 DAP, nodule dry weight, fixed nitrogen, biomass production and pod yield at harvest. The results showed that mid-season drought reduced nodule dry weight, fixed nitrogen, pod yield and increased SCMR. Under drought stress conditions, positive and significant correlations between SCMR and fixed nitrogen with biomass production and pod yield were found. Drought tolerant genotypes had higher SCMR, fixed more nitrogen and achieved higher pod yield than sensitive genotypes. KKU 60 and Tifton 8 were the best genotypes under mid-season drought.

Keywords: Biomass production, nodule dry weight, relationship, SPAD chlorophyll meter reading

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INTRODUCTION

Peanut (*Arachis hypogaea* L.) is the world's 4th most important edible oil crop and 3rd most important source of vegetable protein (CGIAR, 2005). However, over 97.6% of world peanut area and about 95.5% of total production is concentrated in developing countries, predominantly in Asia and Africa, where crop is grown mostly under rain-fed conditions (ICRISAT, 2011). In these regions, low rainfall and prolonged dry spells during crop growth period are main reason for low yields and constraint to peanut production (Kumar, 2007).

Drought stress effects vary according to crop growth stages and duration of stress period (Wright and Nageswara Rao, 1994). However water stress during vegetative stage did not have detrimental effect on peanut yield (Nageswara Rao *et al.*, 1985), and early season or preflowering drought stress followed by re-watering could increase pod yield because peanut has more time for recovery after stress (Puangbut *et al.*, 2009).

The highest water requirement for peanut is during mid-growing season from flowering to pod filling, and at this stage peanut is the most susceptible to drought (Patil and Gangavane, 1990). Water management in mid-season, therefore, becomes extremely important. However, it is unfeasible to invest in irrigation in semi-arid region in Asia and Africa because of limited inputs. Hence, to solve this problem, drought tolerant varieties have been developed, but breeding gained very little success because of complexity of gene controlling drought resistant traits (Serraj and Adu-Gyamfi, 2004). Therefore, it is very important to explore other traits which are easier to determine drought tolerant ability of peanut genotypes.

Reddy *et al.* (2003) reported that development of drought resistant varieties by manipulating genotype variations results in higher water use efficiency (WUE). Peanut genotypes with high WUE under drought conditions are considered to be drought tolerant in terms of total dry matter production (Nautiyal *et al.*, 2002). However, the selection through this process is also difficult or even unsuccessful due to genotypes and environmental variations (Arunyanark *et al.*, 2008).

Several early researches reported that nitrogen fixation in legumes is more sensitive to drought stress than dry matter accumulation (Wery *et al.*, 1994). The effect of water deficit on nitrogen fixation is also larger than that on biomass accumulation (Castellanos *et al.*, 1996; Thomas *et al.*, 2004). Pimratch *et al.* (2008a) revealed that under the long period drought from 21 DAP until to harvest, there were positive relationships between fixed nitrogen and biomass production of the tested peanut genotypes, and relationships were stronger under more severe the drought stress. These open new chance in research to use nitrogen fixation ability as a drought resistance trait of legume, including peanut.

Biological nitrogen fixation from nodule is vitally important for growth and yield of legumes, crop yield often remains low if the legumes don't have nodules (Lindemann and Glover, 2003). Nevertheless, nitrogen fixed is not free because the plant must invest significant amount of energy in the form of photosynthate and other nutritional factors for the bacteria (Lindemann and Glover, 2003). Any stress such as drought, soil salinity or acidity that reduces growth of plant will restrict nitrogen fixation (Naturland, 2000). If a genotype maintains high nitrogen fixation under drought conditions and at the same time it also attains high yield, nitrogen fixation seems to be a drought tolerance character of peanut.

Pimratch *et al.* (2008a) demonstrated this hypothesis. However, they just reported for a long term drought, response of nitrogen fixation in particularly growth stages has not been mentioned. In fact, there were also researches reporting the response of nitrogen fixation when peanut was subjected to early drought stress (Wunna *et al.*, 2009; Puangbut *et al.*, 2011), but the accumulated knowledge so far is not sufficient to understand the responses during mid-season drought. Moreover, during mid-season drought, when nitrogen fixation activity of nodule system is the highest (Nambiar and Dart, 1983), nitrogen fixable will be seriously affected because it has little change to recover (Peña-Cabriales and Castellanos, 1993). Therefore, investigation about response of peanut for nitrogen fixation traits under mid-season drought is extremely necessary.

The objectives of this study were to investigate: (1) the responses of nitrogen fixation of peanut genotypes with different drought resistance levels under mid-season drought condition; (2) relationship between nitrogen fixation traits with agronomic traits and yield of peanut genotypes with different drought resistance levels under mid-season drought conditions.

MATERIALS AND METHODS

The experiment was conducted under field conditions at the Field Crop Research Station of Khon Kaen University, Khon Kaen, Thailand (latitude 16°28' N and longitude 102°48' E, 200 m above mean sea level) during dry-season from November of 2011 to March of 2012. The soil properties were shown in Table 1.

Table 1. Soil properties from experimental site at different depths.

Parameter	Soil depth	
	0-30 cm	30-60 cm
Physical properties		
Sand (%)	93.86	91.87
Silt (%)	4.66	4.02
Clay (%)	1.48	4.11
Texture class	Sand	Sand
Chemical properties		
pH (1:1 H_2O)	6.49	6.60
EC (dS m^{-1})	0.02	0.02
Organic Matter (%)	0.52	0.45
Total N (%)	0.03	0.03
Available P (mg kg^{-1})	58.54	35.99
Exchangeable K (mg kg^{-1})	57.79	43.35
Exchangeable Ca (mg kg^{-1})	340.00	395.00
CEC (c mol kg^{-1})	4.19	4.46

Soil moisture content at field capacity (FC) and permanent wilt point (PWP) were 10.94% and 4.81%, respectively.

Materials and experimental design

Experimental design was a split-plot in randomized complete block design with 4 replications. Two soil moisture regimes, including W1 (well-watered at field capacity) and W2 (mid-season drought by withholding water from 30 to 60 day after planting) were assigned in main plots. Five peanut genotypes (Tainan 9, KS 2, ICGV 98305, Tifton 8 and KKU 60) with different drought tolerance levels were designed randomly in sub-plots.

Tainan 9 is a widely planted cultivar in Thailand with low nitrogen fixation (McDonagh *et al*., 1993), low dry matter production (Vorasoot *et al*., 2003) and susceptible to drought (Jongrungklang *et al*., 2012). KS 2 is a released cultivar in Thailand, and this genotype is susceptible to drought (Jongrungklang *et al*., 2012). ICGV 98305 is a drought resistant line from International Crops Research Institute for the Semi- Arid Tropics (ICRISAT) with high total biomass and pod yield under drought conditions (Nageswara Rao *et al*., 1994, Nigam *et al*., 2005). Tifton 8 is a drought tolerant line from the United States Department of Agriculture (USDA) (Coffelt *et al.,* 1985) with high nitrogen fixation (Pimratch *et al.,* 2010). KKU 60 is a large seed, new recommended cultivar in Thailand with drought tolerance (Jongrungklang *et al*., 2012). The cultivars/lines were planted in the plots with the size of 5.0 x 5.5 m.

Experimental field was prepared before planting by plowing three times. Soil samples were taken to determine the soil physical and chemical properties at the last plowing time. Triple superphosphate and muriate of potash were applied to all of plots at the rates of 24.7 kg P ha^{-1} and 31.1 kg K ha^{-1}, respectively. Amount of fertilizers were calculated by area of plot. The fertilizers were broadcasted thoroughly and incorporated into the soil shortly prior to planting. Seeds of each genotypes were treated with Captan (3a,4,7,7a-tetrahydro-2-[(trichloromethyl)thio]-1H-isoindole-1,3(2H)-dione) at the rate of 5.0 g kg^{-1} seeds before planting to control *Aspergillus niger*. Seeds of Tifton 8 was treated with ethrel (2 chloroethylphosphonic acid) 48% at the rate of 2.0 ml l^{-1} to break seed dormancy before planting.

Three to four seeds per hill were planted by hand with a spacing of 50 cm between rows and 20 cm between hills. *Rhizobium* inoculation was done by applying diluted water commercial peat-based inoculums of *Bradyrhizobium* (mixture of strains THA 201 and THA 205; Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand) at the rate of 13.0 g kg^{-1} seed on rows of peanut after planting, and then water was applied at field capacity. The seedlings were thinned to one plant per hill at 14 days after planting (DAP).

Crop Management

Weeds were controlled by the application of Alachlor (2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide 48%, w/v, emulsifiable concentrate) at the rate of 3.0 l ha^{-1} one day after planting and hand weeding during the remainder of the season. Gypsum ($CaSO_4$) at the rate of 312.5 kg ha^{-1} was applied at 30 DAP to supply calcium for development of pod and seed. Pests and diseases were frequently observed and controlled when they occurred. Carbosulfan (2-3-dihydro-2,2-dimethyl-7-benzofuranyl (dibutylamino) thio) methylcarbamate 20% w/v, water soluble concentrate) at the rate of 2.5 l ha^{-1} and Methomyl (S-methyl-N-[(methylcarbamoyl)oxy] thioacetimidate 40% soluble powder) at the rate of 1.0 kg ha^{-1} were used as insecticides to control thrips and mite.

At the pegging stage, Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate 3% granular) was used as a pesticide at the rate of 18.0 kg ha^{-1} to protect the crop from the soil insects such as subterranean ants (*Dorylus orientalis* Westwood). After the pegging stage, Chlorothalonil (2,4,5,6-tetrachloroisphthalonitrile) at the rate of 2.0 kg ha^{-1} was used to control rust and late leaf spot. Carbosulfan at 2.5 l ha^{-1} was applied regularly along the crop life cycle to control the vector of PBNV (Peanut Bud Necrosis Virus).

Irrigation

Subsurface drip irrigation system (Super typhoon®; Netafim Irrigation Equipment and Drip Systems, Tel Aviv, Israel) with distance of 20 cm between emitters was installed with a spacing of 50 cm between drip lines at 10 cm below the soil surface midway between peanut rows to supply water to the crop. Water was supplied as soon as sowing, and soil content was maintained at field capacity for 0-60 cm of depth until 30 DAP for whole experimental area.

At moisture stress plots, water supplement was paused during period from 30 to 60 DAP, and then re-watering until harvest. At well-watered plot, soil moisture was maintained uniformly throughout crop life around field capacity. Total amount of irrigation water, applied for each plot, was calculated as the sum of crop water requirement (ET_{crop}) and surface evaporation (SE), which were calculated following the methods described by Doorenbos and Pruitt (1992) and Singh and Russell (1981), respectively.

Data collection

Climatic parameters

Climatic parameters including relative humidity (%), water evaporation (mm/day), rainfall (mm/day), maximum and minimum air temperature (ºC) and solar radiation (MJ/m^2/day) were recorded daily from sowing until harvest by a weather station located near by the experimental field.

Soil moisture content

Neutron probe method was used to monitor soil moisture changes by a Neutron Soil Moisture Meter (Type I.H. II SER, N^o N0152, Ambe Diccot Instruments Co. Ltd., England), which can measure soil moisture volume fraction from aluminum access tubes. This was conducted weekly from planting date to harvest with depths in 30, 60 and 90 cm in each sub-plot.

Plant water status

Plant water status was determined by leaf relative water content (RWC) at 30, 45, 60, 75 and 90 DAP, using one leaflet of second fully expanded leaf from the top of main stem of 5 sample plants from each sub-plot at 10:00-12:00 am on clear sky and sunny day (Kramer, 1980). Leaflets from each sub-plot were put into individual vial with a rubber stopper and then sealed with paraffin and keeping suddenly in ice box to prevent moisture loss. The samples were taken to laboratory as soon as possible. Fresh weights of the leaflets were recorded in the laboratory, and the samples were then soaked immediately in distilled water under dim-light and controlled temperature at 24-26^{o}C. At 8 hours after soaking, the saturated weight of leaflets was determined. After that, samples were put into paper bags and oven dried at 80^{o}C for 48 hours or until constant dry weight. Finally, RWC was calculated as follows:

RWC = [(fresh weight – dry weight) / (saturated weight – dry weight)] x 100.

SPAD chlorophyll meter reading

Five plants from each sub-plot were selected randomly to determine SPAD chlorophyll meter reading (SCMR) at 75 DAP. SCMR was recorded by a SPAD chlorophyll meter (Minolta SPAD-502 meter, Tokyo, Japan) on leaflets of second fully expanded leaf from the top of main stem of sample plant between 10.00 am to noon.

Nodule dry weight, biomass production, pod yield and harvest index

At harvest, 10 plants from each sub-plot were taken randomly by digging them. Root samples were used to determine nodule dry weight, while remained parts (shoots and pods) were used to determine biomass and pod yield. Root samples were washed in tap water, and then nodules were removed from roots by hand. Nodules were oven dried at 80 °C for 48 hours or until constant weight was reached to determine dry weight. Pods from each plot were separated and air-dried to approximately 8 % moisture content to determine pod dry weight. The dry weights of shoots were weighted after drying in a hot-air oven at 80 °C for 48 hours or until constant weight was attained. Biomass production was calculated as follows:

Biomass production = shoots dry weight + pods dry weight.

The harvest index (HI) was also calculated as follows:

HI = pod weight / biomass production.

Nitrogen fixation

At harvest, ten sample plants were randomly taken from each sub-plot to determine total nitrogen content. After roots were removed, plant samples were oven-dried at 80 ºC until constant weights were attained, and then dried samples were ground at 70 °C for 2 hour and transferred to desiccators till the temperature of sample was reduced to room temperature. Plant sample was taken randomly to digest by Micro-kjedahl digestion method (Black, 1965), and nitrogen content in sample was determined according to the automated indophenols method (Schuman *et al.*, 1973) by reading on a Flow Injection Analyzer model 5012 (Tecator Inc., Hoganas, Sweden). Total nitrogen content in plant was converted according to biomass production. Nitrogen fixation was calculated by the N-difference method using non-nodulating line as reference plant. This method has been proven in previous studies as an effective method in determining nitrogen fixation (McDonagh *et al*., 1993; Phoomthaisong *et al*., 2003).

Nitrogen fixed by each genotype was calculated using the formula:

Total N-fixed = Total N of each genotype – Total N of the non-nodulating line

Drought tolerance index (DTI) for all traits was calculated as follows:

DTI = value in drought stress condition/value in well-watered condition.

Data Analysis

The data were subjected to analysis of variance according to a split-plot design using MSTAT-C package (Bricker, 1989). Data of each water regime and drought tolerance index of each trait were analyzed according to a randomized complete block design (RCBD), and Least Significant Difference (LSD) was used to compare means (Gomez and Gomez, 1984). Correlation coefficients between nitrogen fixation traits to biomass production, pod yield, harvest index and their drought tolerant indexes were calculated based on n = 20 (5 genotypes x 4 replications) to assess the relationships.

RESULTS

Meteorological conditions

The experiment was conducted under dry season. The total rainfall received during the growing season was approximately 42 mm at 9-10 DAP (8 mm) and 79-81 DAP (34 mm). There was no rainfall received during mid-season drought period. Daily air humidity and daily evaporation ranged from 60.0 to 95.0% and from 2.2 to 9.7 mm/day, respectively (Figure 1a). The daily average air temperature fluctuated from 17.5 to 31.5^{o}C during the growing season, while solar radiation ranged from 12.4 to 23.8 MJ/m^{2}/day (Figure 1b)

Soil moisture content and plant water status

There were large differences in soil moisture contents at 30 cm in depth between 2 water regimes during the drought period, especially before re-watering at 60 DAP (Figure 2a). The differences had downward trends at deeper levels, and became similar at depth of 90 cm (Figure 2b, 2c). The results indicated a good control of the soil moisture content.

As can be seen from the Figure 2d, the significant variation in RWC between water regimes only occurred at 60 DAP. In fact, RWC at field capacity remained around 97.0% from 30 DAP to 90 DAP. Meanwhile, RWC at mid-season drought decreased to just 94.6% at 60 DAP, and then the relative water content was recovered after re-watering.

Effect of mid-season drought and response of peanut genotypes for traits associated with nitrogen fixation

Statistical analysis showed significant differences between water regimes and among peanut genotypes for traits related to nitrogen fixation, including SCMR, nodule dry weight and fixed nitrogen (Table 2). In fact, drought caused significant decreases in nodule dry weight and amount of nitrogen fixation, whereas SCMR increased significantly when peanut genotypes subject to drought condition (Table 3). Interactions between water regimes and genotypes were non-significant for all traits (Table 2).

There were significant differences among genotypes for SCMR at both water regimes. Under well-watered condition, SCMR values ranged from 32.9 to 45.8. Drought tolerant genotypes (KKU 60 and Tifton 8) had the highest SCMR, whereas a drought sensitive genotype (Tainan 9) had the lowest. Under mid-season drought condition, SCMR ranged from 37.2 to 52.3. Drought tolerant genotype (KKU 60) still had the highest SCMR (52.3), whereas drought sensitive genotypes (Tainan 9 and KS 2) had the lowest SCMR values (38.2 and 37.2, respectively). Peanut genotypes were statistically different for DTI for SCMR. However, the different seemed to be between drought sensitive genotypes (Tainan 9 and KS 2).

Peanut genotypes were significantly different for nodule dry weight under both well-watered and drought conditions. Under well-watered condition, Tifton 8 had higher nodule dry weight than did KKU 60 and Tainan 9, but it was not significantly different from another. Under drought stress condition, KKU 60, Tifton 8 and KS 2 had nodule dry weight higher than did Tainan 9 and ICGV 98305. Differences among peanut genotypes for DTI for nodule dry weight were also significant. Tifton 8 and ICGV 98305 seemed to have DTI for nodule dry weight lower than did KS 2 and KKU 60.

Peanut genotypes were significantly different for fixed nitrogen under both water regimes. Under well-watered condition, nitrogen fixations of peanut genotypes ranged from 1.48 to 2.75 (g plant^{-1}). ICGV 98305 and Tifton 8 had higher fixed nitrogen than did Tainan 9 and KS 2. Under stress condition, KKU 60, ICGV 98305 and Tifton 8 had significantly higher fixed nitrogen than Tainan 9 and KS 2. However, the peanut genotypes were not statistically different for DTI for fixed nitrogen.

Effects of mid-season drought and response of peanut genotypes for biomass production, pod yield and harvest index

Mid-season drought reduced biomass production, pod yield and harvest index, but the reductions were not significant except for pod yield (Table 2 and Table 3). Peanut genotypes were significantly different for biomass production, pod yield and harvest index. However, the interactions between water regimes and genotypes were not significant for all traits.

Although the difference between water regimes was not significant for biomass production, peanut genotypes were significantly different for biomass production at both water regimes. Under well-watered condition, ICGV 98305 had the highest biomass production, whereas other genotypes were rather similar. Under mid-season drought, ICGV 98305 had biomass production higher than did Tainan 9 and KS 2, and biomass production of Tifton 8 was higher than that of Tainan 9. However, the differences among genotypes for drought tolerant index (DTI) for biomass production were not significant.

Peanut genotypes were also significantly different for pod yield under both water regimes. KKU 60 and Tifton 8 had higher pod yield than did Tainan 9 and KS 2 at both well-watered and drought stress conditions. Meanwhile, ICGV 98305 was significant higher than Tainan 9 only. The difference for DTI for pod yield was only significant between Tifton 8 and Tainan 9.

Under well-watered condition, KKU 60 had the highest harvest index, while Tifton 8, ICGV 98305 and Tainan 9 had harvest index higher than KS 2. Under stress condition, KKU 60 and Tifton 8 had harvest index higher than did other genotypes. The differences among peanut genotypes for DTI for harvest index were significant. ICGV 98305 and Tifton 8 had DTI for harvest index higher than did Tainan 9 and KS 2.

Relationship between nitrogen fixation traits with biomass production, pod yield and harvest index

Under well-watered condition, pod yield was significantly correlated with SCMR (r = 0.76**) and fixed nitrogen (r = 0.54*). The results might indicated that SCMR (high chlorophyll) and fixed nitrogen contributed to pod yield under well-watered. The correlation coefficients between fixed nitrogen and biomass production was highly significant ($r = 0.84^{**}$) and much stronger than the correlation coefficient between fixed nitrogen and pod yield (r = 0.54*). The results might indicate that, under well-watered condition, fixed nitrogen contributed more to biomass production than pod yield.

Under drought condition, nodule dry weight had positive correlations with DTI for biomass production (r = 0.47*), whereas SCMR had positive correlations with biomass production (r = 0.46*), pod yield (r = 0.80**) and harvest index (r = 0.83**). It is interesting that the correlations between SCMR with pod yield and harvest index under drought were much stronger than the correlation between SCMR with biomass production. In terms of fixed nitrogen, correlations between fixed nitrogen with biomass production and with pod yield were positive and strongly significant (r = 0.88** and r = 0.82**, respectively). Meanwhile, DTI for pod yield and harvest index also had positive correlation with fixed nitrogen (r = 0.59* and r = 0.48*, respectively).

DISCUSSION

Results showed that mid-season drought affected most characters under investigation except for biomass production and harvest index. Mid-season drought reduced nodule dry weight, fixed nitrogen and pod yield. Under drought, the concentration of nitrogen in plant tissues was lower than under normal condition. The reason for this could be that nitrogen fixation was more sensitive to drought than biomass production (Wery *et al.*, 1994). The findings in this study agreed with previous observations under long drought period condition in term of nodule dry weight (Pimratch *et al.*, 2008a), nitrogen fixation (Pimratch *et al.*, 2008b; Pimratch *et al.,* 2010) and pod yield (Vorasoot *et al.*, 2003). Previous researches, Naveen *et al.* (1992) and Wright *et al.* (1999) also found similar results in reduction of pod yield under mid-season drought.

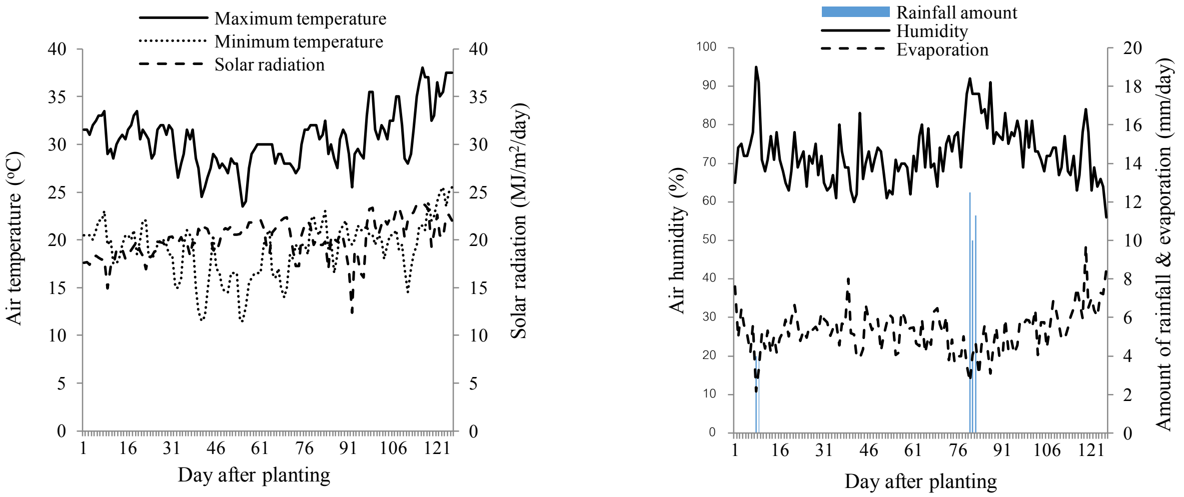

Figure 1. Air temperature and solar radiation (a), air humidity, rainfall and evaporation (b) during dry season 2011-2012.

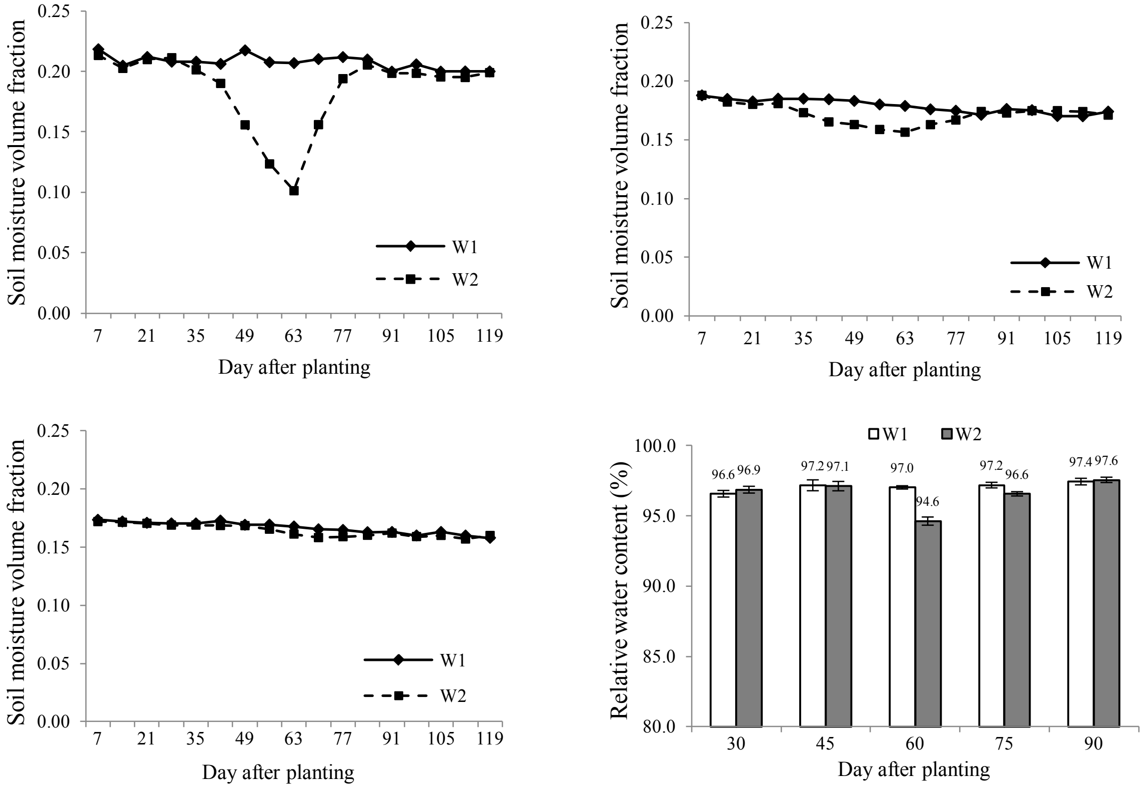

Figure 2. Soil moisture volume fractions at depth of 30 cm (a), 60 cm (b), 90 cm (c), and relative water content (d) at well-watered (W1) and mid-season drought (W2) during dry season 2011-2012.

Table 2. Mean square for SPAD chlorophyll meter reading (SCMR), nodule dry weight (NDW), fixed nitrogen, biomass production, pod yield and harvest index.

Source of variance	SCMR	NDW	Fixed nitrogen	Biomass production	Pod yield	Harvest index
Replication (R)	10.62	0.01	0.05	822.36	30.35	0.002
Water regimes (W)	126.38**	0.12**	1.09*	1086.81	308.114**	0.011
Error R*W	3.66	0.00	0.07	147.73	4.32	0.002
Genotype (G)	239.57**	0.24*	2.27**	2668.49**	659.12**	0.036**
W*G	12.90	0.01	0.06	139.90	17.40	0.002
Error R*W*G	4.90	0.01	0.16	228.65	42.60	0.003
CV (R*W)	4.64	10.73	13.76	12.39	6.45	12.49
CV (R*W*G)	5.36	22.38	20.87	15.41	20.23	16.63

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

Table 3. SPAD chlorophyll meter reading (SCMR), nodule dry weight (NDW), fixed nitrogen, biomass production, pod yield and harvest index.

Genotype	SCMR			NDW (g plant^{-1})			Fixed nitrogen (g plant^{-1})		
	W1	W2	DTI	W1	W2	DTI	W1	W2	DTI
KKU60	45.8^{a}	52.3^{a}	1.14^{ab}	0.38^{b}	0.37^{a}	0.97^{a}	2.20^{ab}	1.92^{a}	0.89^{a}
ICGV98305	38.8^{b}	43.0^{b}	1.12^{ab}	0.48^{ab}	0.28^{b}	0.58^{b}	2.75^{a}	2.15^{a}	0.83^{a}
Tainan9	32.9^{c}	38.2^{c}	1.16^{a}	0.39^{b}	0.28^{b}	0.70^{ab}	1.48^{c}	1.11^{b}	0.76^{a}
Tifton8	43.5^{a}	44.7^{b}	1.03^{ab}	0.57^{a}	0.38^{a}	0.66^{b}	2.51^{a}	2.24^{a}	0.92^{a}
KS2	36.6^{b}	37.2^{c}	1.02^{b}	0.45^{ab}	0.41^{a}	0.96^{a}	1.50^{bc}	1.36^{b}	0.90^{a}
Mean	39.5^{B}	43.1^{A}	1.09	0.45^{A}	0.34^{B}	0.77	2.09^{A}	1.76^{B}	0.86

Genotype	Biomass production (g plant^{-1})			Pod yield (g plant^{-1})			Harvest index		
	W1	W2	DTI	W1	W2	DTI	W1	W2	DTI
KKU60	104.4^{b}	96.2^{abc}	0.93^{a}	47.7^{a}	39.3^{a}	0.86^{ab}	0.44^{a}	0.40^{a}	0.94^{ab}
ICGV98305	136.2^{a}	113.2^{a}	0.79^{a}	35.8^{b}	31.0^{ab}	0.87^{ab}	0.27^{b}	0.28^{b}	1.05^{a}
Tainan9	86.5^{b}	72.4^{c}	0.85^{a}	26.0^{c}	17.7^{c}	0.68^{b}	0.31^{b}	0.25^{b}	0.81^{b}
Tifton8	104.6^{b}	102.7^{ab}	1.00^{a}	38.8^{b}	37.4^{a}	1.00^{a}	0.37^{ab}	0.36^{a}	1.00^{a}
KS2	84.9^{b}	80.0^{bc}	0.94^{a}	26.9^{c}	22.2^{bc}	0.83^{ab}	0.32^{b}	0.25^{b}	0.79^{b}
Mean	103.3	92.9	0.90	35.8^{A}	29.5^{B}	0.85	0.34	0.31	0.90

W1 (well-watered at field capacity condition), W2 (mid-season drought condition). DTI (drought tolerance index). Different small letters in the same column show significance between genotypes at $P < 0.05$ by LSD. Different capital letters in the same row show significance between water conditions at $P < 0.05$ by LSD.

Table 4 Correlation between traits related to nitrogen fixation with biomass production, pod yield and harvest index (n = 20).

Traits	SCMR		Nodule dry weight		Fixed nitrogen	
	W1	W2	W1	W2	W1	W2
Biomass production	0.32	0.46*	0.08	0.21	0.84**	0.88**
DTI- biomass production	0.09	0.18	0.28	0.47*	-0.19	0.38
Pod yield	0.76**	0.80**	0.10	0.35	0.54*	0.82**
DTI- pod yield	0.23	0.22	0.46*	0.32	0.13	0.59*
Harvest index	0.56*	0.82**	0.02	0.24	-0.02	0.48*
DTI- Harvest index	0.28	0.26	0.18	-0.19	0.49*	0.44

* and ** = significant at $P < 0.05$ and $P < 0.01$, respectively. W1 (well-watered at field capacity condition), W2 (mid-season drought condition). DTI (drought tolerance index)

In contrast, peanut genotypes responded to mid-season drought by increase of SCMR. SCMR increased as a result of increasing chlorophyll content per unit area when plants were subjected to drought stress (Nageswara Rao and Wright, 1994). Under different drought conditions, long term drought (Jongrungklang *et al.*, 2008) and early season drought (Wunna *et al.*, 2009) also increased SCMR. Therefore, it seems to be that nitrogen fixation traits as nodule dry weight, SCMR and fixed nitrogen were appropriate to evaluate effects of drought in general and mid-season drought in particular.

Nageswara Rao *et al.* (1989) found a poor relationship between the yield potential and the sensitivity of genotypes to mid-season drought and suggested a possibility to identify genotypes with high yield potential and relatively low sensitivity to mid-season droughts. In this study, there were differences in nitrogen fixation traits, biomass productions, pod yields and harvest indexes among peanut genotypes under both well-watered and mid-season drought conditions. Similarly, in earlier work, peanut genotypes showed significant differences in nodule dry weigh, biomass and nitrogen fixation under long term drought and field capacity conditions (Pimratch et al., 2008a, b; Pimratch *et al*., 2010).

In this study, the interaction effects between genotype and water regime were low and not significant for all traits. In the earlier work, Wunna *et al.* (2009) did not found genotypic difference among peanut genotypes for nodule dry weight, biomass production, pod yield and harvest index under field capacity and early season drought, but they found similar interaction effects. Phenotypic variations contributed to a large portion of total variations in nitrogen fixation traits, biomass production and pod yield. The genotype with high nodule dry weight, SCMR, nitrogen fixation, biomass production and pod yield under well-watered conditions also have high values for all these traits under mid-season drought. High potential under well-watered conditions is important for high performance under drought conditions.

Differences in water regimes were more pronounced at the soil depths of 30 cm during the period 50 to 65 DAP, while other periods and soil depths were just mild stress. In this case, differences in root distributions among peanut genotypes might explain differential responses to drought. Tainan 9 and KS 2 with high root length density (RLD) in upper layer (0-30 cm in soil depth) and middle layer (30-60 cm) but low RLD in lower layer (60-90 cm) (Jongrungklang *et al.,* 2012) were sensitive to both mild and severe stresses. In contrast, ICGV 98305 (high RLD in middle layer), KKU 60 (high in middle and lower layer) and Tifton 8 (high RLD in lower layer) (Jongrungklang *et al.*, 2012) were slightly affected by drought. This could be elucidated why under drought stress condition, tolerant genotypes can maintain SCMR, fixed nitrogen amount, biomass production and pod yield better than sensitive genotypes.

In this study, the correlation between fixed nitrogen and biomass production was significant, but correlation between nodule dry weight and biomass production was not significant. In previous research, Pimratch *et al.* (2004) reported that under well-watered condition, total fixed nitrogen content and nodule dry weight were significant and positively correlated with total biomass and fixed nitrogen was correlated with pod weight per plant. Present and previous findings were similar in general, and the small difference could be possibly due to difference in experiment conditions. This experiment used rhizobium inoculation, while Pimratch *et al.* (2004) did not.

Under mid-season drought condition, fixed nitrogen had positive and significant correlations with biomass production, pod yield and DTI for pod yield. This indicated that genotypes with high level for drought tolerance gained high yield under drought stress because they could fix and maintain high nitrogen in plant. It can be seen that amounts of nitrogen fixed by drought tolerant genotypes such as KKU 60, ICGV 98305 and Tifton 8 were higher than those fixed by drought sensitive genotypes under both normal condition and drought conditions.

CONCLUSION

Drought during the time of mid-season reduced nitrogen fixation, nodule dry weight and pod yield, but increased SCMR. Correlations between nitrogen fixation traits, including SCMR and fixed nitrogen with biomass production and with pod yield were positive and significant. Drought tolerant genotypes fixed more nitrogen and achieved higher yield than sensitive genotypes. KKU 60 and Tifton 8 were the best genotypes for high nitrogen fixation traits under mid-season drought.

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