



Association of transpiration efficiency with N₂ fixation of peanut under early season drought

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Abstract

Peanut is grown mostly in rainfed areas where drought is a recurring problem. Peanut genotypes with high transpiration efficiency (TE) use less water and produced yield better under drought conditions. Specific leaf area and SPAD chlorophyll meter reading are used as surrogate traits for TE. N₂ fixation (NF) is also used as a surrogate trait for yield under drought. The objective of this study was to demonstrate the relationship between TE and NF and their contributions to yield under early season drought (ESD). A field experiment was conducted in a split-plot design with four replications for two seasons. Early drought (1/3 available water from emergence to 40 days after emergence) and irrigated control were assigned in main-plots, and 12 peanut genotypes were assigned in sub-plots. Data were recorded for TE, NF and pod yield at harvest. ESD increased TE and NF. KK 60-3 had high TE and also had high NF under drought conditions. Under drought conditions, TE was strongly and positively correlated with N₂ fixation. Hence, high NF might contribute to high TE under ESD conditions. KK 60-3 is a superior genotype for its ability to maintain high N₂ fixation, and it could improve TE under ESD conditions. Improvement of NF combined with high TE would have contributed to higher pod yield under drought conditions. It was apparent that enhanced NF also increased TE and pod yield. Thus, selecting for improved NF under ESD conditions may be an effective indirect selection technique to improve yield under drought conditions.

Keywords: *Arachis hypogaea* L.; Water deficit; Nitrogen fixation; Pod yield.

Introduction

Peanut (*Arachis hypogaea* L.) is an important cash crop grown largely under rainfed conditions in the semi-arid tropics, where the crop is usually affected by drought stress at various stages of crop growth (Nageswara Rao et al., 1985). Severity of drought stress depends on the stages of crop development and the duration of stress period (Wright and Nageswara Rao, 1994). Yield of peanut is generally reduced by drought (Nautiyal et al., 1999; Songsri et al., 2008). However, in some instances the pre-flowering drought (PFD) has been shown to increase yield (Puangbut et al., 2010).

In Southeast Asia, most of peanut production area is under rainfed conditions (Jogloy et al., 1992). Most of peanut is grown in the monsoon during late April and early May (Reddy et al., 2003). In this situation, drought is a recurring during pre-flowering phase. Therefore, selection of superior genotypes for high yield by exposing the peanut to PFD might be a promising strategy to improve pod yield.

Optimum use of stored soil water, high transpiration efficiency (TE) and high partitioning of biomass into economic yield under limited water conditions is an ultimate goal of any drought research (Krishnamurthy et al., 2007; Abbasi and Sepaskhah, 2011). Because peanut is usually affected by insufficient water supply, improvement of TE would be a promising strategy to cope with episodes of drought. Traits associated with TE have been reported such as SPAD chlorophyll meter reading (SCMR) (Nageswara Rao et al., 2001; Sheshshayee et al., 2006), specific leaf area (SLA) (Nageswara Rao and Wright, 1994; Wright et al., 1994) and root dry weight (Songsri et al., 2009; Puangbut et al., 2009b).

The improvement of TE can be achieved indirectly by selecting surrogate traits such as SLA (Wright et al., 1994; Nageswara Rao and Wright, 1994), carbon isotope discrimination (Hubick et al., 1986; Farquhar et al., 1988; Wright et al., 1994) and SCMR (Nageswara Rao et al., 2001; Sheshshayee et al., 2006). Recent reports have demonstrated that root dry weight and SLA were important traits related to WUE and should be useful selection criteria for high WUE under long term drought (Songsri et al., 2009).

Drought generally reduces N_2 fixation (NF) (Pimratch et al., 2008; Reddy et al., 2003; Serraj et al., 1999). However, drought stress during the pre-flowering stage has been shown to increase NF (Puangbut et al., 2010; Venkateswarlu et al., 1990). Recent study reported that the correlation between NF and yield under PFD was positive and significant and the maintenance of NF under drought stress could be an important adaptive mechanism in sustaining yields (Puangbut et al., 2010).

A better understanding of the traits associated with TE such as the ability of NF to increase nutrient uptake and maintain high photosynthetic capacity should be useful in improving TE and their contribution to yield under drought conditions. However, very limited information has been available for the relationship between TE and NF under ESD conditions. Selection of superior peanut genotypes for ability to maintain high NF might help to improve TE under ESD. It is expected that selection of genotypes for high NF coupled with high TE might help to improve yield under ESD. A better understanding of their contribution to yield is important for peanut breeding. Therefore, the objective of this study was to investigate the relationship between NF and TE under ESD and their contributions to yield under ESD conditions.

Materials and Methods

Experimental design and treatments

The field experiment was conducted for two seasons in the rainy season during June to October 2005 and in the dry season during December 2005 to April 2006 at the Field Crop Research Station of Khon Kaen University located in Khon Kaen province, Thailand (16° 28' N, 102° 48' E, 200 masl). Two seasons were selected in stead of two years because year variation would be minimized by irrigation, while seasonal variation was still large.

Twelve peanut genotypes were used in this study. Eight elite drought resistant Spanish type varieties (ICGVs 98300, 98303, 98305, 98308, 98324, 98330, 98348 and 98353) were donated from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). Tifton-8, a Virginia-type drought resistant line, was kindly provided by the United State Department of Agriculture (USDA) (Coffelt et al., 1985). KK 60-3 (Virginia type, drought susceptible and high NF) and Tainan 9 (Spanish type and low NF) are the cultivars commercially released in Thailand (Vorasoat et al., 2003; McDonagh et al., 1993). The lines from ICRISAT were selected because of their drought tolerance characteristics (Nageswara Rao et al., 1992; Nigam et al., 2003; Nigam et al., 2005). A non-nodulating line (Non-nod) was also included as a reference in determining NF ability (McDonagh et al., 1993).

In the rainy season, rainout shelters were available and used when necessary, but in the dry season the experiment was carried out under field conditions without rainout shelter. The soil type is Yasothon series (Yt: fine-loamy; siliceous, isohypothermic, Oxic Paleustults). A split-plot in a randomized complete block design with four replications was used in both seasons. Main-plots were two water treatments [field capacity (FC) and 1/3 available water (1/3 AW)], and sub-plot treatments were 12 peanut genotypes. Plot sizes were 2.5×2.1 m in the rainy season and 3×3 m in the dry season with a spacing of 30 cm between rows and 10 cm between plants.

Crop management

Lime at the rate of 625 kg ha⁻¹ was incorporated into the soil during land preparation. Basal fertilizers as triple superphosphate at the rate of 122 kg ha⁻¹ and potassium chloride at the rate of 62 kg ha⁻¹ were then incorporated into the soil, but nitrogen fertilizer was not applied. A pre-emergence herbicide, alachlor (2-chloro-2', 6'-diethyl-N-(methoxymethyl) acetanilide 48%, w v⁻¹, emulsifiable concentrate) at the rate of 3 l ha⁻¹ was applied after planting. Seeds were treated with captan at the rate of 5 g kg⁻¹. The seeds of two Virginia-type cultivars (KK 60-3 and Tifton-8) were separately treated with ethrel (2 ml l⁻¹) to break seed dormancy before planting. Rhizobium inoculation was applied by diluting 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) in water and applied to soil immediately after planting. The seedlings were thinned to one plant per hill at 7 days after emergence (DAE).

Gypsum was applied at pegging stage at the rate of 312 kg ha⁻¹. Carbofuran (2, 3-dihydro-2, 2-dimethylbenzofuran-7-ylmethylcarbamate 3% granular) was applied to the soil at pod setting stage to control soil insects. Standard management practices were followed with appropriate pest and disease management practices as described by Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand.

A subsurface drip-irrigation system (Super Typhoon[®], Netafim Irrigation equipment & Drip systems, Israel) was installed with a spacing of 30 cm between driplines and 20 cm between emitters. The driplines were installed 10 cm below the soil surface mid-way between rows and a pressure valve and water meter were fitted to ensure controlled application of water to the treatments. Soil moisture was initially supplied with a water field capacity (FC) to a depth of 20 cm and to facilitate uniform emergence. At 8 days after last irrigation, soil moistures for the ESD treatment was allowed to gradually decline until it

reached the soil moisture level of 1/3 available water (AW) at 20 DAE, the soil moisture was maintained at this level until 40 DAE when re-watering was applied to the crop at FC moisture level and the soil moisture was maintained at FC until harvest. The irrigated treatment was maintained at FC moisture level until harvest.

Soil moisture content at FC and permanent wilting point (PWP) were determined as 11.3% and 4.9%, respectively, using pressure plate method. Soil moisture content for 1/3 AW treatment was estimated to be 6.9% as a proportional value between FC and PWP. In maintaining the specified soil moisture levels, water was added to the respective main-plots by subsurface drip-irrigation based on crop water requirement and surface evaporation which were calculated following the methods of Doorenbos and Pruitt (1992) and Singh and Russell (1981), respectively.

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

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

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

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

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

Weather parameters

Weather data were obtained from the nearest meteorological station (Figure 1). In the rainy season, there was a 230 mm rainfall during the ESD period (0-40 DAE) and total rainfall during the crop was 743.7 mm (Figure 1a). However, the rainfall event during the ESD conditions could be ignored because the crop was protected by rainout shelters during drought period. In the dry season, there was no interference from rain during the ESD conditions but there was a 24.4 mm rainfall during 66 to 71 DAE (Figure 1c). The seasonal means of maximum and minimum air temperatures ranged between 31.5 and 26.1 °C in the rainy season and 32.6 and 18.8 °C in the dry season (Figure 1b, d). Daily pan evaporations ranged from 1.42 to 7.52 mm in the rainy season and 2.78 to 9.92 mm in the dry season (Figure 1a, c). The seasonal means of solar radiations ranged from 14.3 MJ m⁻² d⁻¹ in the rainy season and 15.9 MJ m⁻² d⁻¹ in the dry season, respectively (Figure 1b, d).

Soil moisture status

Soil moistures were measured by gravimetric method at planting and harvest at the depths of 0-5, 25-30 and 55-60 cm. The measurement at planting was for calculating the

correct amount of water to be applied to the crop, and the measurement at harvest was for calculating the water use of the crop. The soil water status was also monitored at 7-day intervals using a neutron moisture meter (Type I.H. II SER. N° N0152, Ambe Diccot Instruments Co. Ltd., England).

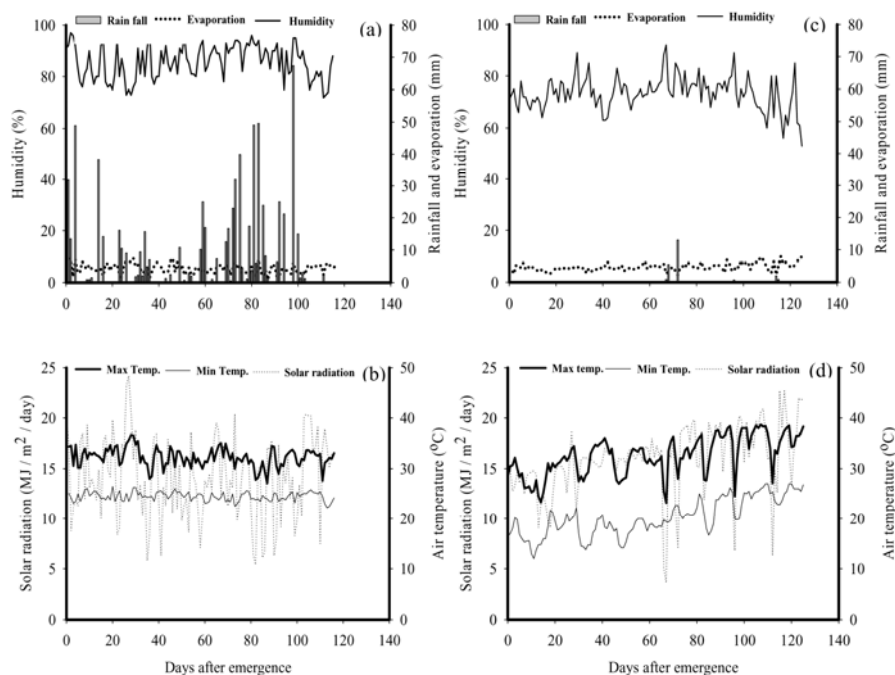


Figure 1. Rain fall, humidity (RH), evaporation (E_0), maximum and minimum temperature and solar radiation in the rainy season (2005) (a, b) and in the dry season (2005/06) (c, d) at the meteorological station of Khon Kaen University.

Pod yield and Biomass

Pod yield and biomass at final harvest was determined from a harvested area of 1.8 m² in the rainy season and 3.84 m² in the dry season. The pods were removed before taking shoot fresh weight in the field, and one kg of random sample of shoots was oven-dried at 80 °C for 48 hours and dry weight was measured. Shoot dry matter content was then calculated and used in determining shoot dry weight for a plot. Pod yield was measured after air drying to 8% moisture content. Biomass was computed by the following formula:

$$\text{Biomass} = \text{shoot dry weight} + \text{pod dry weight}$$

N_2 fixation

NF was measured at final harvest. For each plot, five plants were sampled. The total N content of plant (shoot, seed and shell) was analyzed by micro-kjedahl digestion (Black,

1965). Elemental nitrogen was measured by flow injection analyzer model 5012 (Tecator, 1984). Nitrogen fixed by each genotype was calculated as:

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

The N in non-nod genotype represented the uptake of mineral N from soil (McDonagh et al., 1995). Earlier studies have shown that the N-difference method used in this study was as effective as the ^{15}N isotope dilution method in determining NF (McDonagh et al., 1993; Bell et al., 1994; Phoomthaisong et al., 2003).

Transpiration efficiency

Transpiration (T) was calculated using the methods described by Doorenbos and Pruitt (1992), using the relationship as follows:

$$T = I + P + (M_i - M_f) - (E_s + D)$$

where, T = crops transpiration between period *i* and *f*, I = irrigation amount, P = rainfall, M_i = starting soil moisture at time *i*, M_f = soil moisture at the time *f* (soil moisture was measured by auger method), E_s = soil evaporation and D is drainage, which is assumed as close to zero.

TE for each treatment was calculated as above ground biomass including pods (BIO) divided by T.

$$TE = \text{BIO (g)} / T \text{ (kg)}$$

Statistical analysis

Individual analysis of variance was performed for each character in each experiment. Error variances for the two seasons were tested for homogeneity by Bartlett's test (Hoshmand, 2006). Combined analyses of variance were undertaken for characters with homogeneous variances, and Duncan's multiple range test (DMRT) was used to compare means.

Simple correlation was used to determine the relationship between TE and NF measured under irrigated and ESD treatments and simple correlations were computed to determine the relationship between TE and NF with pod yield under ESD treatments.

Results

Monitoring of soil moisture

Soil moisture was measured using a neutron moisture meter at 7-day intervals to harvest (Figure 2). The results showed reasonable management of soil moistures. A clear distinction between soil moisture levels were noted at 30 and 60 cm of soil depth. Under the ESD treatment, soil moisture was reduced during water deficit (0-40 DAE). However, after re-watering, soil moisture was increased to a range similar to that for irrigated treatment until at harvest in both seasons.

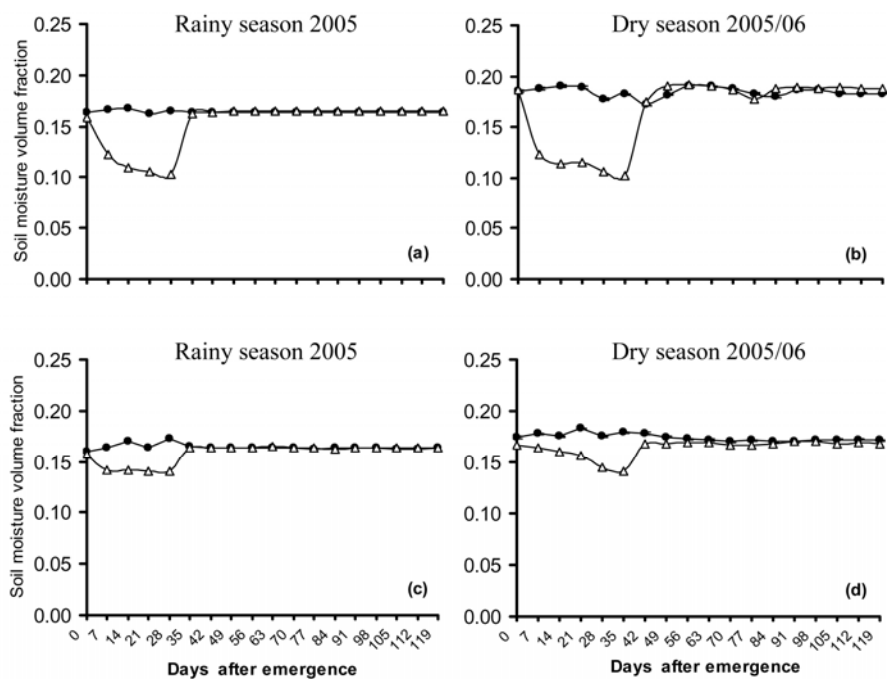


Figure 2. Soil moisture volume fraction in two available soil water regimes [field capacity (FC), ●; and 1/3 available water (AW), Δ] at 30 cm (a, b) and 60 cm (c, d) of the soil level in the rainy season 2005 and dry season 2005/06).

Pod yield

Pod yield differed significantly among genotypes with pod yield ranged from 2619 to 3375 kg h⁻¹ under the irrigated conditions (Table 1). However, under the ESD conditions, pod yield ranged from 2535 to 4000 kg h⁻¹ resulting in an increase of 37% over the irrigated control. Whilst 9 out of 11 genotypes showed positive yield response to ESD, but ICGV 98305 and ICGV 98353 showed negative response under the ESD.

Genotypic variability of N₂ fixation and TE

Significant differences among peanut genotypes were found for NF and TE under well-watered and ESD conditions (Table 1). Drought at early growth stages significantly increased NF and TE. KK 60-3 had the highest NF and TE under well-watered and ESD conditions. Under ESD conditions, KK 60-3 had high NF and TE (247.6 kg N ha⁻¹, and 2.9 g kg⁻¹, respectively). The lowest NF and TE was observed in ICGV 98353 under two water regimes (138.0 kg N ha⁻¹ and 1.7 g kg⁻¹ for well-watered treatment, 165.0 kg N ha⁻¹ and 2.0 g kg⁻¹ for ESD treatment).

Table 1. Pod yield, N₂ fixation and transpiration efficiency (TE) of 11 peanut genotypes under irrigated and early season drought (ESD) conditions.

Genotypes	Pod yield		N ₂ fixation		TE	
	(kg ha ⁻¹)		(kg N ha ⁻¹)		(g kg ⁻¹)	
	Irrigated	ESD	Irrigated	ESD	Irrigated	ESD
ICGV 98300	3035 ^b	3775 ^b	181.7 ^c	220.9 ^b	2.1 ^{bcd}	2.8 ^a
ICGV 98303	3375 ^a	4000 ^a	171.1 ^d	188.5 ^d	2.1 ^{bcd}	2.3 ^{cd}
ICGV 98305	3175 ^b	2885 ^{cd}	171.5 ^d	176.8 ^e	1.9 ^{ef}	2.1 ^{ef}
ICGV 98308	2825 ^{bc}	3025 ^c	156.8 ^{ef}	183.2 ^{de}	2.0 ^{de}	2.2 ^{de}
ICGV 98324	3010 ^b	3400 ^{bc}	165.0 ^{de}	170.0 ^f	1.9 ^{ef}	2.4 ^{bc}
ICGV 98330	2775 ^c	3160 ^c	166.1 ^{de}	169.3 ^f	2.1 ^{bcd}	2.4 ^{bc}
ICGV 98348	2660 ^c	3150 ^c	148.6 ^f	187.1 ^{de}	1.9 ^{ef}	2.2 ^{de}
ICGV 98353	2835 ^{bc}	2535 ^d	138.0 ^g	165.7 ^f	1.7 ^f	2.0 ^f
Tainan 9	2850 ^{bc}	3675 ^b	183.9 ^c	207.1 ^c	2.2 ^{bc}	2.5 ^b
KK 60-3	2619 ^c	3476 ^{bc}	205.8 ^a	247.6 ^a	2.5 ^a	2.9 ^a
Tifton-8	2802 ^{bc}	3339 ^c	184.4 ^{bc}	208.9 ^c	2.3 ^{ab}	2.8 ^a
Mean	2924	3429	170.3	193.2	2.1	2.4

Mean in the same column with the same letters are not significantly different by Duncan's Multiple Range Test (DMRT) (at P<0.05).

Relationship between NF and TE and their contribution to yield under ESD

There was a consistently strong and positive correlation between NF and TE under irrigated and ESD conditions. Under ESD conditions, the highest positive correlation was found between NF and TE ($r=0.87$; $P\leq 0.01$). KK 60-3 and ICGV 98303 had high NF which may have contribution to their high TE under ESD conditions (Figure 3).

There was a positive correlation between NF and TE with pod yield in the ESD conditions but not in the irrigated treatment. Under ESD conditions, the highest positive correlation was found between NF and pod yield ($r=0.64$; $P\leq 0.05$) (Figure 4). Similarly, there was a positive correlation between TE and pod yield under ESD ($r=0.70$; $P\leq 0.05$). The highest for NF and TE was observed in KK 60-3 and ICGV 98303 under ESD (Figure 4).

Discussion

The present study supported previous findings that imposition of PFD followed by adequate water supply can result in higher yield compared to irrigated conditions (Puangbut et al., 2009a; Puangbut et al., 2010; Nageswara Rao et al., 1988). This increase in yield due to a flush of flower after re-watering and could set more pegs and mature pods resulted in higher pod yield at harvest (Nautiyal et al., 1999; Nageswara Rao et al., 1988). In addition, Puangbut et al. (2010) reported that NF was an important factor contributing to yield advantage under PFD. Therefore, selection of peanut genotypes for high NF could result in improve yield under ESD.

Identification of the physiological traits associated with drought resistance of plant under drought conditions has been a goal of new breeding programs. TE is a drought resistance trait which can conserve soil moisture and enhance WUE and makes contribution to yield under unpredictable or limiting soil water available conditions.

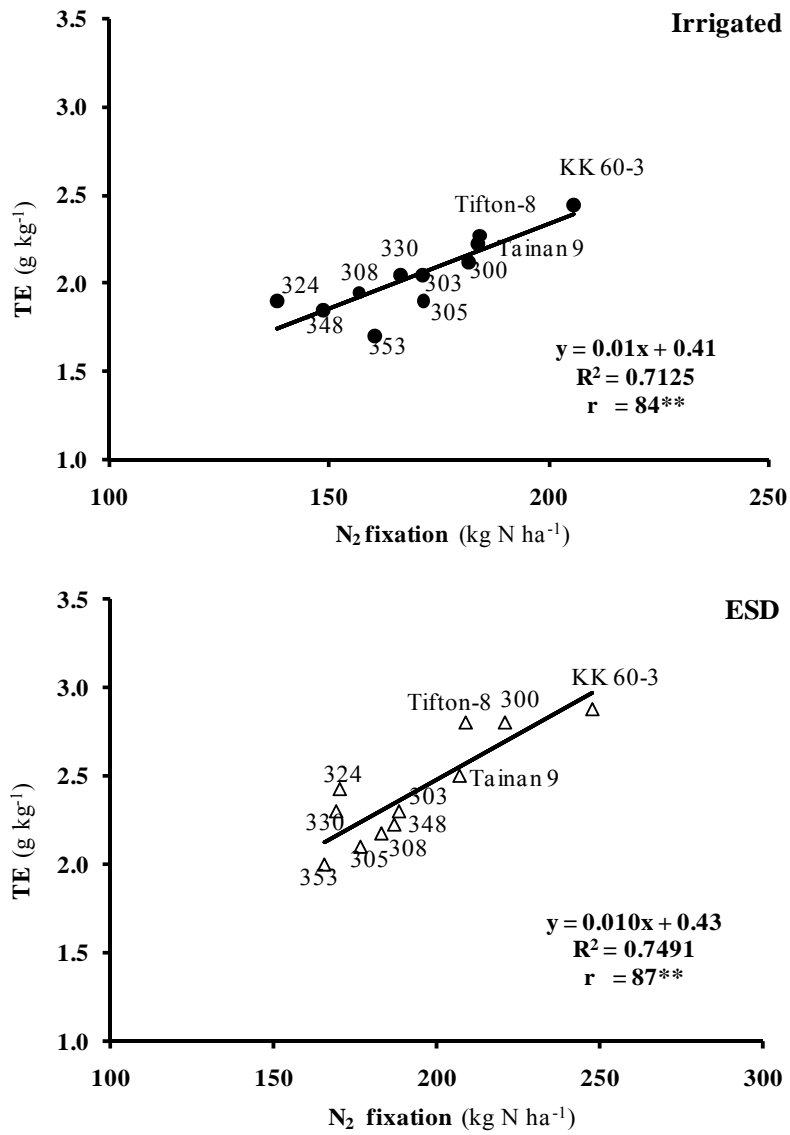


Figure 3. Relationship between N₂ fixation and transpiration efficiency (TE) of 11 peanut genotypes under irrigated and early season drought (ESD) treatments.

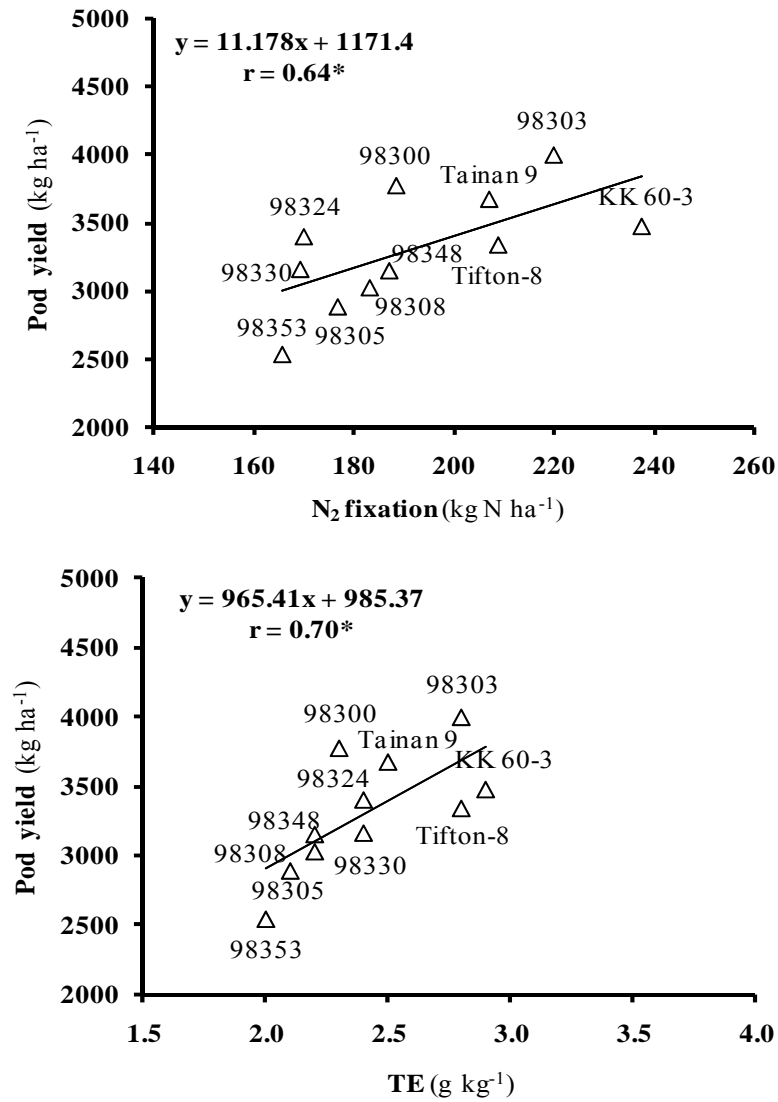


Figure 4. Relationship between N₂ fixation and transpiration efficiency (TE) and pod yield of 11 peanut genotypes under early season drought (ESD) treatments.

An understanding of the traits associated with TE such as NF to increase nutrients uptake and maintain high photosynthetic capacity coupled with enhanced biomass should be useful in improving TE under drought conditions. This information should provide a better understanding on how genotypes could achieve high TE coupled with enhance pod yield under drought, and could have important implications on breeding for drought resistance in peanut.

Peanut showed significant differences in traits associated with TE. KK 60-3 had the highest NF and also had the highest TE under ESD conditions (Table 1). The ability of peanut genotypes with high NF and root dry weight to enhance nutrients and water uptake may explain their relatively high TE under ESD conditions (Puangbut et al., 2009b; Puangbut et al., 2010). The present study indicated that NF may be a major component contributing to TE under ESD conditions (Figure 3). It was apparent that NF could be useful as a selection criterion for enhancing TE in peanut.

It was apparent that an improved pod yield under early season drought was associated with increases NF and TE (Figure 4). NF has been shown to be strongly correlated with leaf area (Puangbut et al., 2010). It could be hypothesized that peanut genotypes with high leaf area have more photosynthetic efficiency and hence potential for greater assimilation under drought stress. While TE could be enhanced by increase in root growth which would allow plants to absorb additional water and nutrients from deeper soils layers and promote overall plant growth which in turn contributes to yield (Puangbut et al., 2009b).

The results revealed that an increased NF under the ESD followed by re-watering was associated with higher TE (Figure 3). Previous study indicated that NF was correlated with biomass (Pimratch et al., 2008) and pod yield (Puangbut et al., 2010; Bell et al., 1994). It may be possible to improve TE coupled with high yield in peanut by selecting for high NF under ESD conditions. Puangbut et al. (2010) indicated that NF had a relatively low G×E interactions and could be amenable to selection in breeding programs aimed at improving drought resistance in peanut. The information on NF under ESD conditions may have application in genetic improvement of drought resistance in peanut.

Conclusions

This study demonstrated that ESD is not seriously detrimental to yield. NF and TE increased with ESD. In addition, genotypes with high NF could maintain relatively high TE under drought conditions. KK 60-3 had high NF and TE under ESD conditions. The results indicated that the genotypes with high NF and also had high TE under ESD. The present study revealed that an improved pod yield under ESD was associated with increases NF and TE. Our results suggested that there may be good possibilities to increase TE coupled with high yield of peanut by selecting for improved NF under ESD conditions.

Acknowledgments

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