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# Association of stomatal conductance and root distribution with water use efficiency of peanut under different soil water regimes

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**Abstract**

Drought is a major abiotic stress limiting crop productivity worldwide especially in rain-fed areas. Plant physiologists and crop breeders have aimed to better understand the mechanisms underlying drought resistance to increase the success in breeding for drought resistant crops. The objectives of this study were to (i) determine the effects of different soil water availability on stomatal behavior and water use efficiency (WUE) of diverse peanut genotypes and ii) to investigate associations between surrogate physiological traits for drought tolerance. Eleven peanut genotypes and three soil moisture levels (field capacity, 2/3 available water and 1/3 available water) were assigned in a split plot design with four replications for two experiments. WUE, stomatal traits and root distribution (indicated by root length density, RLD, in the soil layers at 0-40 cm and 40-100 cm depths) were measured at 37, 67 and 97 days after sowing (DAS). Drought reduced stomatal aperture and stomatal conductance but increased WUE. Differential responses among peanut genotypes were observed for WUE, and stomatal conductance. ICGV 98300 and Tifton 8 had high stomatal conductance and WUE under moderate water stress. Stomatal conductance and %RLD $_{(40\text{-}100cm)}$ play an important roles in increasing WUE under mild drought stress.

**Keywords:** Drought tolerance, Gas exchange, Stomatal behavior, Water stress, WUE.
**Abbreviation:** FC - Field capacity; DMRT - Duncan's multiple range test; PWP - Permanent wilting point; WUE - Water Use Efficiency; RLD - root length density; RWC – relative water content.

## Introduction

Peanut production is mostly in rain-fed areas of the arid and semi-arid tropics, where unpredictable and insufficient rainfall is prevalent. Drought is a recurring problem which limits peanut yields (Redy et al., 2003; Songsri et al., 2008b, 2009a) and also increases aflatoxin contamination (Nageswara et al., 2002; Holbrook and Stalker, 2003; Girdthai et al., 2010). Peanut genotypes with improved drought resistance and/or higher water use efficiency (WUE) are desirable. The development of peanut cultivars with resistance to drought and more efficient use of water offers the best long term and cost effective solution to the drought problem. Selection for yield *per se* under drought conditions has been used to breed crops for drought resistance (Araus et al., 2008; Kumar et al., 2008). However, progress from this strategy has been slow due to genotype by environmental interactions. More rapid progress may be achieved by selecting for physiological traits associated with yield performance, such as the ability of the roots to take up water, reduced water loss by more responsive stomatal closure and increased WUE under drought conditions (Ober et al., 2005). However, stomatal closure to reduce water loss also limits $CO_2$ uptake. Crops can avoid drought conditions by increasing water uptake if sufficient water is available within the root zone (Wright and Nageswara Rao, 1994; Taiz and Zeiger, 2006) and enhancing WUE by altering stomatal behavior (Passioura, 2002). Root distribution, root length density (RLD), specific leaf area (SLA) and SPAD chlorophyll meter reading (SCMR) have been proposed as useful criteria for selecting for improved drought adaptive traits (Matsui and Singh, 2003; Aranyanark et al., 2009; Puangbut et al., 2011). Water use efficiency is a trait associated with drought avoidance mechanisms (Anyia and Herzog, 2004). WUE is positively associated with SCMR, but it is negatively associated with SLA (Sheshshayee et al., 2006; Songsri et al., 2009b). WUE is also closely related to stomatal conductance in faba bean (Ricciardi, 1989). Stomatal behavior regulates water loss and transpiration efficiency (TE) under drought stress (Darwish and Fahmy, 1997), and therefore influences WUE. The close association of drought adaptive traits and WUE has been reported in other crops including rice (Kobata et al., 1996), sorghum (Tolk and Howell, 2003) and Ponderosa pine (Zhang et al., 1997). Under conditions with limited available soil water, the balance between the increase in water uptake by deeper roots and the reduction in water loss by stomatal control is critical to maintaining high crop productivity. Unfortunately, this information for the association of stomatal conductance and root distribution with WUE under drought stress has not been available for peanut. To better understand the association of physiological traits to WUE, further research is required. The aims of the current investigation were to (i) determine the effects of different available soil water levels on stomatal behavior and WUE among diverse peanut genotypes and (ii) to investigate the associations between surrogate physiological traits for drought tolerance.

**Table 1.** Water use efficiency (WUE), stomatal density, stomatal conductance, and stomatal aperture of 11 peanut genotypes grown under different available soil water: field capacity (FC), 2/3 available water (2/3 AW) and 1/3 available water (1/3 AW).

| Genotype | WUE (g kg$^{-1}$) | | | | | | Stomatal density (stomata mm$^{-2}$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | FC | | 2/3 AW | | 1/3 AW | | FC | | 2/3 AW | | 1/3 AW | |
| ICGV 98300 | 1.76 | b | 1.87 | a | 1.90 | ab | 290 | ab | 275 | cde | 269 | cde |
| ICGV 98303 | 1.56 | cd | 1.58 | c | 1.85 | abc | 252 | cd | 248 | ef | 240 | ef |
| ICGV 98305 | 1.53 | cd | 1.66 | bc | 1.71 | bc | 226 | d | 260 | def | 228 | f |
| ICGV 98308 | 1.48 | d | 1.69 | bc | 1.67 | c | 234 | d | 313 | ab | 249 | def |
| ICGV 98324 | 1.61 | bcd | 1.75 | abc | 1.83 | abc | 291 | ab | 321 | a | 311 | ab |
| ICGV 98330 | 1.50 | d | 1.68 | bc | 1.80 | bc | 269 | bc | 286 | a-d | 278 | cd |
| ICGV 98348 | 1.56 | cd | 1.76 | ab | 1.69 | bc | 303 | a | 294 | a-d | 299 | abc |
| ICGV 98353 | 1.68 | bc | 1.76 | ab | 1.69 | bc | 323 | a | 308 | abc | 292 | bc |
| Tainan 9 | 1.61 | bcd | 1.59 | bc | 1.77 | bc | 219 | d | 238 | f | 227 | f |
| KK 60-3 | 1.77 | ab | 1.76 | ab | 2.04 | a | 302 | ab | 261 | def | 294 | bc |
| Tifton -8 | 1.94 | a | 1.89 | a | 1.86 | abc | 316 | a | 285 | bcd | 327 | a |
| *Mean* | 1.64 | | 1.73 | | 1.80 | | 275 | | 281 | | 274 | |

| Genotype | Stomatal conductance (mmol m$^{-2}$ s$^{-1}$) | | | | | | Stomatal aperture (*u*m) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | FC | | 2/3 AW | | 1/3 AW | | FC | | 2/3 AW | | 1/3 AW | |
| ICGV 98300 | 677 | a | 269 | | 240 | a | 5.38 | | 4.63 | a | 3.25 | |
| ICGV 98303 | 475 | d | 237 | | 210 | ab | 4.00 | | 2.63 | bc | 2.75 | |
| ICGV 98305 | 486 | cd | 240 | | 204 | ab | 4.25 | | 3.25 | bc | 2.75 | |
| ICGV 98308 | 531 | bcd | 205 | | 163 | ab | 5.38 | | 3.00 | bc | 2.63 | |
| ICGV 98324 | 464 | d | 191 | | 132 | b | 4.13 | | 3.13 | bc | 2.13 | |
| ICGV 98330 | 560 | bc | 212 | | 209 | ab | 4.50 | | 3.25 | bc | 2.38 | |
| ICGV 98348 | 474 | d | 199 | | 150 | ab | 4.63 | | 3.25 | bc | 2.88 | |
| ICGV 98353 | 571 | b | 226 | | 149 | b | 5.13 | | 3.75 | ab | 2.63 | |
| Tainan 9 | 452 | d | 200 | | 164 | ab | 4.38 | | 2.50 | c | 2.75 | |
| KK 60-3 | 500 | bcd | 258 | | 146 | b | 4.88 | | 3.75 | ab | 2.63 | |
| Tifton -8 | 472 | d | 276 | | 188 | ab | 4.75 | | 2.88 | bc | 3.13 | |
| *Mean* | 515 | | 228 | | 177 | | 4.67 | | 3.27 | | 2.72 | |

Mean in the same column with the same letters are not significantly different by DMRT at p<0.05.

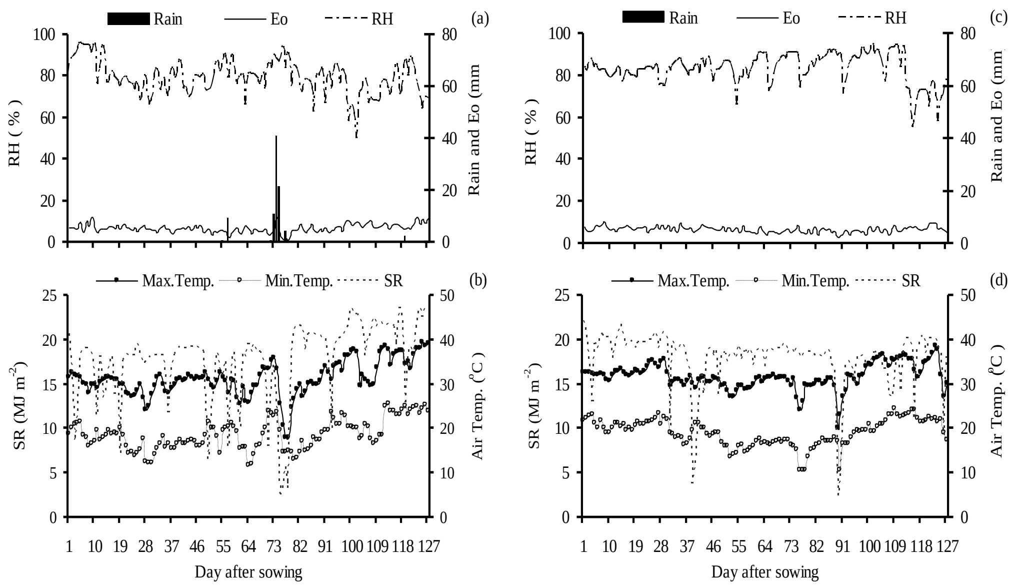


**Fig. 1.** Rainfall, evaporation ($E_O$), relative humidity (RH), maximum and minimum air temperature (Max and Min Temp.) and solar radiation (SR) in 2003-04 (a, b) and 2004-05 (c, d).

## Results

### *Meteorological conditions, soil moisture status and variation of plant water status*

Although the experiment was conducted in the dry period, there was a downpour of 71 mm during 73-75 DAS in 2003/04 (Fig. 1). The downpour caused the soil moisture contents of the drought treatments to be high for one week, whereas there was no rainfall in 2004/05 (Fig. 2). In both years, the different irrigation treatments showed distinctly different soil moisture contents in the top soil layers except for the rainfall period in 2003/04 (Fig. 2). Soil moisture contents at 60 cm were more similar among water treatments, and there was no difference at 90 cm. The average maximum and minimum air temperature ranged between 31.0$^{o}$C and 18.0$^{o}$C in 2003/04 (being lower during 1-45 DAS) and 32.0$^{o}$C and 19.0$^{o}$C in 2004/05. Daily evaporation ranged from 0.8 to 9.9 mm in 2003/04 and 2.2 to 8.3 mm in 2004/05

(Fig. 1). The average solar radiation was similar in both years (17.6 MJ $m^{-2}d^{-1}$ in 2003/04 and 17.7 MJ $m^{-2}d^{-1}$ in 2004/05). Leaf relative water content (RWC) at 97 DAS was highest at FC (92%) followed by 2/3 AW (88%) and 1/3 AW (77%), respectively. ICGV 98300, ICGV 98324, ICGV 98330 and Tifton 8 maintained high RWC when droughted (2/3 AW and 1/3 AW) whereas Tainan 9 exhibited lower RWC under 2/3 AW and 1/3 AW.

### *Variability of water use efficiency (WUE) and stomatal parameters*

Drought generally increased water use efficiency, but reduced stomatal conductance and aperture (Table 1). Drought stress had a greater effect on stomatal conductance and stomatal aperture than stomatal density. Stomatal conductance decreased by 56% and 66% at 2/3 AW and 1/3 AW respectively, whereas stomatal aperture decreased by30% and 42% respectively. Stomatal density was higher at 2/3 AW than at FC and 1/3 AW. WUE and stomatal behaviour differed significantly between genotypes under both well-watered and droughted conditions (Table 1). Tifton 8 and ICGV 98300 had high WUE under droughted conditions, and WUE was highest at 2/3 AW. In contrast, ICGV 98303 and ICGV 98308 showed low WUE under drought conditions, whilst ICGV 98353 and Tainan 9 had low WUE under both non-stressed and stressed conditions. However, there was no clear relationship between stomatal density and WUE in peanut in response to drought (Fig. 3).

### *Traits contributing to WUE*

Multiple regression analysis evaluated the contributions of stomatal density, stomatal conductance, stomatal aperture and root length density to WUE under FC, 2/3 AW and 1/3 AW conditions (Table 2). A significant regression was observed at 2/3 AW only, explaining 94.45% of the total variation in WUE. Stomatal conductance accounted for 54.18% of total variation in WUE, followed by stomatal density (20.00%) and root length density at 0-40 cm and 40-100 cm (11.04 and 5.59%), whereas the contribution of stomatal aperture was not significant. Thus stomatal conductance was more sensitive to drought conditions than other physiological traits.

### *Correlation of physiological traits and WUE*

The relationship between stomatal density and WUE was positive but not significant, and a similar relationship was observed for stomatal conductance and WUE (Fig. 3a and b). The relationship between WUE and root length density was negative and significant in the upper soil layers (RLD $_{0\text{-}40\ cm}$; r = -0.70, p<0.05) whereas strongly positive and significant in the lower soil layers (RLD $_{40\text{-}100\ cm}$; r = 0.70, p<0.05) (Fig. 3c and d). Under mild drought conditions, the genotypes with highest WUE were Tifton-8 and ICGV 98300. Although there were no statistical differences, both genotypes had numerically high stomatal conductance. ICGV 98300 also had the highest root length density at 40-100 cm, although its root length density at 0-40 cm was lowest (Data not presented). Tifton-8 had intermediate root length density at 0-40 cm and 40-100 cm. Tainan 9 had the lowest stomatal density, stomatal conductance and root length densities at 0-40 cm and 40-100 cm. Genotypes ICGV 98324, ICGV 98308 and ICGV 98353 with high stomatal density had low stomatal conductance.

## Discussion

Genotypes that increase WUE when exposed to drought may be exhibiting a potential mechanism of drought avoidance mediated by stomatal closure (Gilbert et al., 2011) and/or deeper root systems (Songsri et al., 2009b). However, two peanut genotypes (ICGV 98300 and Tifton-8) showed high WUE at 2/3 AW and also had high stomatal conductance and root length density within the lower soil layers. High stomatal conductance was sustained in these genotypes due to their deeper root systems, which contributed to higher WUE. The increase in WUE under drought stress found in this study was similar to those reported in common bean (Costa et al, 2000) and cowpea (Anyia and Herzog, 2004). In peanut, previous studies found that stomatal conductance was closely related to leaf water status (Bennet at al., 1984). Hence, Stomatal conductance and (deep) root systems may be characteristic traits to consider in the development of genotypes with increased drought tolerance. (Reynolds et al., 2007; Lopes and Reynolds, 2010; Martin-Vertedor and Dodd, 2011). In general, drought stress influenced the balance between stomatal conductance and internal leaf capacity for photosynthesis. Stomatal conductance was found to be closely linked to gas-exchange and water status in $C_3$ plants (Van de Water et al., 1994). In contrast, Sekiya and Yano (2008) reported that soil water content did not have a significant effect on stomatal index under drought stress. On the one hand, Black et al., (1985) had been reported that stomatal conductance was highest under mild drought (2/3 AW) in peanut. In this study, differential response of peanut genotypes for WUE was observed at 2/3 AW, and therefore the water uptake by roots which supports high stomatal conductance under drought. In the present study, stomatal conductance was more important than root length density in determining WUE under moderate drought (2/3 AW) than under FC or 1/3 AW. Our results showed genotypic variation in stomatal conductance under well-watered conditions, however, drought at 1/3 AW severely impaired the function of stomata. Regression analysis of WUE failed to explain the variation at FC and 1/3 AW, whereas the regression at 2/3 AW could explain variation in WUE. This observation suggested that plants extracted sufficient water from the soil under well-watered conditions, so the drought avoidance mechanisms such as stomatal closure and deeper root penetration were not important to improving WUE. Under severe drought, the stomata closed all day because the plants conserved water for survival under extreme stress conditions (Soukup et al., 2004). Roots at lower soil depth (40-100 cm) are more important under moderate drought (2/3 AW) as they extract additional water from soil, as indicated by the positive and significant correlation between RLD $_{40\text{-}100\ cm}$ and WUE. In contrast, RLD at the soil surface (0-40 cm) was negatively and significantly correlated with WUE, indicating that high RLD at the soil surface is not a surrogate physiological trait for drought tolerance. The peanut genotype with the largest deeper root system (RLD $_{40\text{-}100\ cm}$) ICGV 98300 exhibited high WUE and was previously reported as having the largest deeper root system (RLD 40-100 cm) (Sogsri et al., 2008). Improvement of crops with deeper root systems is a viable means to maximize water use (Barnes, 1983). The peanut genotypes with larger root systems in the deeper soil layers were more tolerant to drought, with increased soil water uptake capacities (Songsri et al., 2009b). Insights from the present study should help plant breeder in devising strategies for developing peanut with improved drought tolerance.

**Table 2.** Contributions of stomatal density, stomatal aperture, stomatal conductance, percent of root length density (%RLD) at upper soil layer (0-40cm) and lower soil layer (40-100cm) to WUE under field capacity (FC), 2/3 available water (2/3 AW) and 1/3 available water (1/3 AW).

| | Explained by regression (%) | | |
|---|---|---|---|
| | FC | 2/3 AW | 1/3 AW |
| *Regression* | *64.61* | *94.45*** | *36.75* |
| Stomatal density | 40.95 | 20.00** | 0.99 |
| Stomatal aperture | 15.38 | 3.64 | 0.44 |
| Stomatal conductance | 0.01 | 54.18** | 0.95 |
| %RLD(0-40cm) | 1.91 | 11.04* | 32.15 |
| %RLD(40-100cm) | 6.37 | 5.59* | 2.21 |

*, ** significant at p<0.05 and significant at p<0.01, respectively.

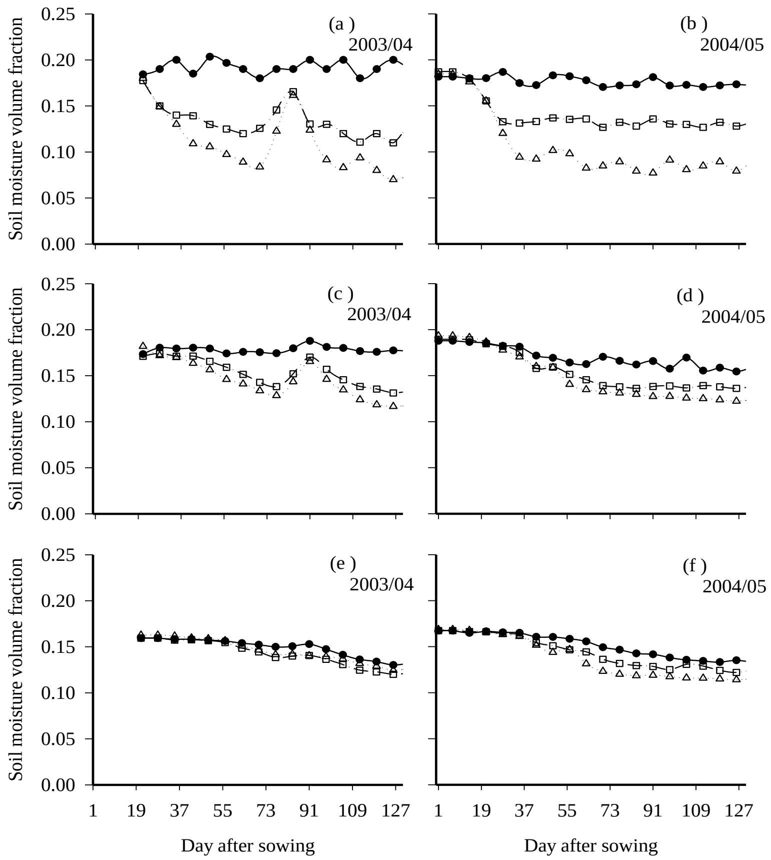


**Fig 2.** Soil moisture volume fraction as measured by neutron moisture meter for three soil water regimes (FC, ●; 2/3 AW, □ and 1/3 AW, Δ) at 30 cm (a, b), 60 cm (c, d) and 90 cm (e, f) of the soil level during the 2003/04 and 2004/05 seasons.

**Material and methods**

***Experimental conditions and materials***

The experiment was conducted at the Field Crop Research Station of Khon Kaen University, Khon Kaen province, Thailand (lat 16° 28´ N, long 102° 48´ E, 200 m above sea level) during November 2003 to March 2004, and repeated during October 2004 to February 2005. Soil pH of 6.16–6.30, total nitrogen 0.03-0.04 %, available phosphorus 35.32-45.84 ppm, and potassium 57.27 ppm. Peanut genotypes used in this study consisted of eight drought resistant lines obtained from ICRISAT (ICGV 98300, ICGV 98303, ICGV 98305, ICGV 98308, ICGV 98324, ICGV 98330, ICGV 98348 and ICGV 98353) with high total biomass and pod yield in screening tests under drought conditions (Nigam et al., 2003, 2005), one drought resistant line (Tifton-8) with a large root system (Coffelt et al., 1985) received from the United States Department of Agriculture, and two cultivars that are commonly grown in Thailand (KK 60-3 and Tainan 9). KK 60-3 is sensitive to drought for pod yield, and Tainan 9 has a low dry matter production (Vorasoot et al., 2003, 2004). A split plot design with four replications was used for two years. Soil moisture treatments [field capacity (FC), 2/3 available water (2/3 AW) and 1/3 available water (1/3 AW)] were assigned in main plots, and 11 peanut genotypes were arranged in subplots. Rainfall, evapoaration ($E_o$), relative humidity (RH), maximum and minimum air temperature and solar radiation (SR) were recorded daily from sowing until harvest were obtained using a meteorological station located approximately 30 m from the experimental site.

***Crop management***

Soil preparation was done by ploughing the field three times to 30 cm depth. Lime at 625 kg ha$^{-1}$, triple superphosphate at 24.7 kg P ha$^{-1}$ and potassium chloride at 31.1 kg K ha$^{-1}$ was

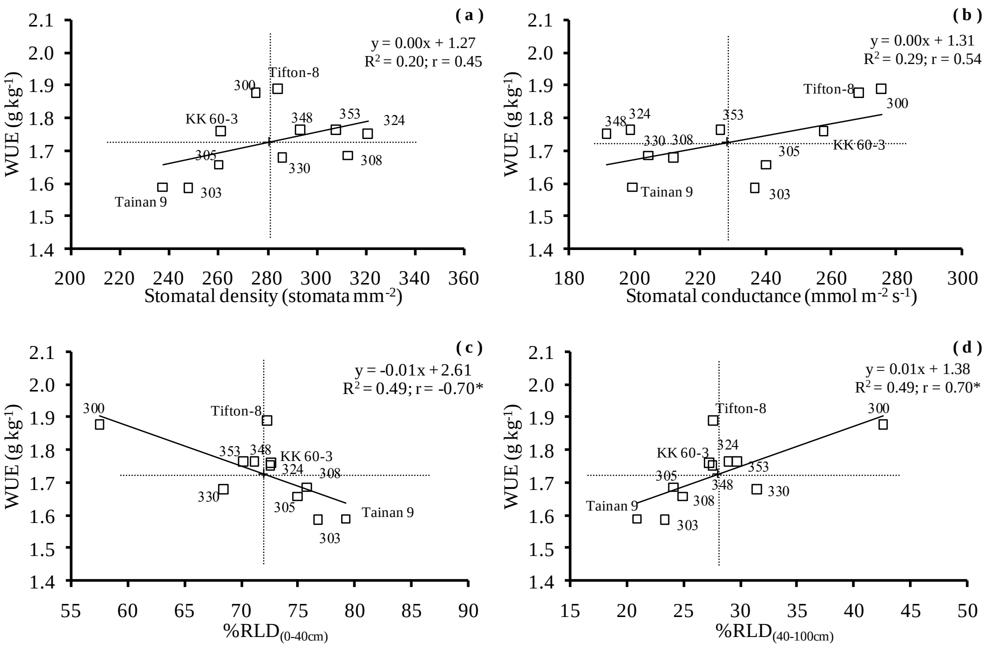


**Fig 3.** Relationship between water use efficiency (WUE) and stomatal density (a), stomatal conductance (b), percent of root length density (%RLD) at upper soil layer (0-40cm) (c), %RLD at lower soil layer (40-100cm) (d) under 2/3 AW of 11 peanut genotypes.

applied prior to planting. Seeds were treated with captan (3a, 4, 7, 7a-tetrahydro-2-[(trichloromethyl)thio]-1H-isoindole-1, 3(2H)-dione) at the rate of 5 gm $kg^{-1}$ seeds and ethrel (2-chloroethylphosphonic acid) 48% at the rate of 2 mL $L^{-1}$ before planting. Three to four seeds were planted per hill and the seedlings were thinned to two plants per hill at 14 days after sowing (DAS). Rhizobium inoculation was done by applying 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 in the evening on the day after sowing. Weeds were controlled by application of alachlor (2-cholro-2', 6'-diethyl-*N*-(methoxymethyl) acetanilide 48%, w $v^{-1}$, emulsifiable concentrate) at the rate of 3 L $ha^{-1}$ at planting and hand weeding during the season. Gypsum ($CaSO_4$) was applied at 45 DAS (312 kg $ha^{-1}$). Carbofuran (2, 3-dihydro-2, 2-dimethylbenzofuran-7-ylmethylcarbamate 3% granular) was applied at the pod setting stage. Pests and diseases were controlled by weekly applications of carbosulfan [2-3-dihydro-2,2-dimethylbenzofuran-7-yl (dibutylaminothio) methylcarbamate 20% w $v^{-1}$, water soluble concentrate] at 2.5 L $ha^{-1}$, methomyl [*S*-methyl-*N*-((methylcarbamoyl) oxy) thioacetimidate 40% soluble powder] at 1.0 kg $ha^{-1}$ and carboxin [5, 6-dihydro-2-methyl-1, 4-oxath-ine-3-carboxanilide 75% wettable powder] at 1.68 kg $ha^{-1}$. A drip-irrigation system (Super Typhoon®; Netafim Irrigation equipment & Drip systems, Tel Aviv, Israel) was installed at 10 cm below the soil surface between peanut rows with a spacing of 50 cm between drip lines to supply water to the crop, and set up with a pressure valve and a water meter to ensure the uniform supply of measured amounts of water to the experiment. Soil moisture was initially maintained at field capacity (93 mm in 60 cm soil depth) until 21 DAS in all treatments to support crop establishment. At 21 DAS, 2/3 AW and 1/3 AW levels of drought stress were imposed by withholding irrigation until soil moisture was reduced to the predetermined levels of 75 and 56 mm in 60 cm depth in 2/3 AW and 1/3 AW treatments, respectively. Stress treatments reached 2/3 AW at 28 DAS and 1/3 AW at 35 DAS, respectively. The 2/3 AW and 1/3 AW water treatments were maintained ± 1% of the predetermined levels until harvest. Water was added to the respective plots by subsurface drip-irrigation based on crop water requirements and surface evaporation for maintaining the specified soil moisture levels, which were calculated following Doorenbos and Pruitt (1992) and Singh and Russell (1981) respectively, and described elsewhere by Songsri et al. (2008a, 2008b). Total crop water use for each water treatment was calculated as the sum of transpiration and soil evaporation. Crop water requirement was calculated using the methods described by Doorenbos and Pruitt (1992):

$$ETcrop = ETo \times Kc, \quad (1)$$

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

$$Es = \beta \times \frac{Eo}{t}, \quad (2)$$

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

### *Soil moisture and plant water status*

Soil moisture was measured by the gravimetric method at planting and harvest, at 0-5, 25-30 and 55-60 cm soil depths. Measurements at planting aimed to calculate the correct amount of water to be applied to the crop, while those at harvest allowed water use of the crop to be calculated. The soil moisture was also measured at 7-day intervals using a neutron probe (Type I.H. II SER. N° N0152, Ambe Didcot Instruments Co. Ltd., Abingdon, Oxon, UK). An aluminum access tube was installed between rows in each plot. Sixteen-second neutron moisture meter readings were made at least weekly from a depth of 0.3 m to 0.9 m at 0.3 m intervals.

Leaf relative water content (RWC) was measured at 37, 67 and 97 DAS. Five leaves of the second fully expanded leaf from the top of the main stem for each plot at 10:00-12:00 h. RWC was recorded using the formula as follows:

$$\text{RWC}(\%) = [\frac{(\text{FW}-\text{DW})}{(\text{TW}-\text{DW})}] \times 100, \qquad (3)$$

Where FW = sample fresh weight, TW = sample turgid weight and DW = sample dry weight.

***Root distribution***

At 97 DAS, roots were sampled using a coring tube (7.60 cm in diameter and 115.00 cm long) as described by Songsri et al. (2008a). Percent root length density (%RLD) was calculated as percentages in the upper (0-40cm) and lower (40-100 cm) soil layers.

***Stomatal characteristics***

At 37, 67 and 97 DAS, stomatal density and aperture were measured on the back of the third fully expanded leaf by smearing the leaves with a solution (3:1 v/v of Collodion (Nitro cellulose solution 4%) and Dimethyl ether after the solution dried. The thin film was peeled from the leaf surface, mounted on a glass slide, immediately covered with a cover slip and sealed. Numbers of stomata and aperture size were measured under a photomicroscope. Stomatal conductance was measured from the second fully expanded leaf using a porometer (Model AP4, Delta-T Devices, Cambridge, UK).

***Calculation of water use efficiency (WUE)***

Evapotranspiration (ET) under varying watering regimes was calculated using the soil water balance equation for the growing season as follows:

$$ET = I + (Mi - Mf) - D - R \qquad (4)$$

Where I is the applied irrigation, M$i$ is the starting soil moisture before sowing, M$f$ is the soil moisture at final harvest (soil moisture was measured by gravimetric method as described above), D is the soil water drainage, and R is the surface runoff. Water drainage and runoff were set to zero.

Water use efficiency (WUE) for each treatment was calculated as above ground biomass including pods (BIO) divided by seasonal evapotranspiration (ET):

$$\text{WUE} = \frac{\text{BIO (g)}}{\text{ET (kg)}} \qquad (5)$$

***Statistical analysis***

Analysis of variance was performed for each characteristic in each experiment. Error variances were tested for homogeneity by Bartlett's test (Hoshmand, 2006) in two seasons. Combined analyses of variance were done. Means were compared by Duncan's multiple range test (DMRT).

Multiple-linear regression was used to determine the relative contribution of %RLD $_{(0\text{-}40cm)}$, %RLD $_{(40\text{-}100cm)}$, stomatal density, stomatal aperture and stomatal conductance to WUE under FC, 2/3 AW and 1/3 AW using the equation (Hoshmand 2006):

$$Y_i = \alpha + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \beta_4 X_{4i} + \beta_5 X_{5i} + \delta_i \qquad (6)$$

Where $Y_i$ is the WUE of genotype i, $\alpha$ is the Y intercept, $X_{1i}$, $X_{2i}$, $X_{3i}$, $X_{4i}$ and $X_{5i}$ are the %RLD$_{(0\text{-}40cm)}$, %RLD$_{(40\text{-}100cm)}$, stomatal density, stomatal aperture and stomatal conductance of genotype i, respectively, $\beta_1$, $\beta_2$, $\beta_3$, $\beta_4$ and $\beta_5$ are the regression coefficients for the independent variables $X_1$, $X_2$, $X_3$, $X_4$ and $X_5$ and $\delta_i$ is the associated deviation from regression. The analysis was carried out 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 most important variable first. The relative contributions of the individual independent variables to WUE under FC, 2/3 AW and 1/3 AW 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.

**Conclusions**

There was limited genetic variation in peanut water use efficiency under fully-irrigated and severe drought conditions, whereas considerable variation (94.45 %) was observed under mild drought stress. The genotypes with high stomatal conductance and deeper root penetration (ICGV 98300 and Tifton 8) could maintain relatively high WUE under drought conditions. Our experiment demonstrated that stomatal conductance and root distribution in deeper soil are important physiological traits related to WUE under mild drought conditions. Therefore, stomatal conductance and root distribution should be used as selection criteria for drought tolerance in peanut varieties.

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**References**

Anyia AO, Herzog H (2004) Genotypic variability in drought performance and recovery in cowpea under controlled environment. J Agron Crop Sci 190: 151–159.

Araus JL, Slafer GA, Royo T, Serret MD (2008) Breeding for yield potential and stress adaptation in cereals. Crit Rev Plant Sci 27: 377–412.

Arunyanark A, Jogloy S, Wongkaew S, Akkasaeng C, Vorasoot N, Wright GC, Rachaputi Rao CN, Patanothai A (2009) Association between aflatoxin contamination and drought tolerance traits in peanut. Field Crops Res 114: 14–22.

Barnes DK (1983) Managing root systems for efficiency water use: breeding plants for efficient water use, In: Taylor HM, Jordan WR, Sinclair TR (Eds), Limitations to efficient water use in crop production. Madison, WI: ASA, pp. 127–135.

Bennett JM, Boote KJ, Hammond LC (1984) Relationship among water potential components, relative water content, and stomatal resistance of field-grown peanut leaves. Peanut Sci 11: 31–35.

Black CR, Tang DY, Ong CK, Solon A, Simmonds LP (1985) Effects of soil moisture stress on the water relations and water use of groundnut stands. New Phytol 100 (3): 313–328.

Coffelt TA, Hammons MM, Branch WD, Mozingo RW, Phipps PM, Smith JC, Lynch RE, Kvien CS, Ketring DL, Porter DM, Mixon AC (1985) Registration of Tifton-8 peanut germplasm. Crop Sci 25: 203.

Costa FMG, Pham TAT, Pimental C, Pereyra RRO, Zuily FY, Laffray D (2000) Differences in growth and water relations among *Phaseolus vulgaris* cultivars in response to induced drought stress. Environ Exp Bot 43: 227–237.

Darwish DS, Fahmy GM, (1997) Transpiration decline curves and stomatal characteristics of faba bean genotypes. Biol Plant 39: 243–249.

Doorenbos J, Kassam AH, (1986) Groundnut. In: Yield Response to Water FAO Irrigation and Drainage Paper No 33. FAO, Rome, pp. 97–100.

Doorenbos J, Pruitt WO (1992) Calculation of crop water requirements. In: Guideline for Predicting Crop Water Requirement FAO Irrigation and Drainage Paper No. 24. FAO, Rome, pp. 1–65.

Gilbert ME, Zwieniecki MA, Holbrook NM (2011) Independent variation in photosynthetic capacity and stomatal conductance leads to differences in intrinsic water use efficiency in 11 soybean genotypes before and during mild drought. J Exp Bot 1–13.

Girdthai T, Jogloy S, Vorasoot N, Akkasaeng C, Wongkaew S, Holbrook CC, Patanothai A (2010) Associations between physiological traits for drought tolerance and aflatoxin contamination in peanut genotypes under terminal drought. Plant Breed 129: 693–699.

Holbrook CC, Stalker HT (2003) Peanut breeding and genetic resources. Plant Breeding Reviews 22: 297–356.

Hoshmand AR (2006) Design of Experiments for Agriculture and the Natural Sciences (2nd). Chapman & Hall/CRC, Boca Raton, Florida.

Kobata T, Okuno T, Yamamoto T (1996) Contributions of capacity for soil water extraction and water use efficiency to maintenance of dry matter production in rice subjected to drought. Jpn J Crop Sci 65: 652–662.

Kumar A, Bernier J, Verulkar S, Lafitte HR, Atlin GN (2008) Breeding for drought tolerance: Direct selection for yield, response to selection and use of drought-tolerant donors in upland and lowland-adapted populations. Field Crops Res 107: 221–231.

Lopes MS, Reynolds MP (2010) Partitioning of assimilates to deeper roots is associated with cooler canopies and increased yield under drought in wheat. Funct Plant Biol 37: 147-156

Martin-Vertedor AI, Dodd IC (2011) Root-to-shoot signaling when soil moisture is heterogeneous: increasing the proportion of root biomass in drying soil inhibits leaf growth and increases leaf abscisic acid concentration. Plant Cell Environ 34: 1164-1175.

Matsui T, Singh BB (2003) Root characteristics in cowpea related to drought tolerance at the seedling stage. Exp Agric 39: 29–38.

Nageswara Rao RC, Singh S, Sivakumar MVK, Srivastava KL, William JH (1988) Effect of water deficit at different growth phase of peanut. II yield response. Agron J 80: 431–438.

Nageswara Rao RC, Wright GC, Krosch S (2002) Management practices to minimise pre-harvest aflatoxin contamination in Australia peanuts. Aust J Exp Agric 42: 595–605.

Nigam SN, Basu MS, Cruickshank AW (2003) Hybridization and description of the trait-based and empirical selection programs. In: Cruickshank AW, Rachaputi NC, Wright GC, Nigam SN (Eds), Report of a Workshop, Breeding for Drought Resistant Peanuts, 25─27 February 2002. ICRISAT Centre Andhra Pradesh, India, ACIAR, Canberra, Australia, pp. 15–17.

Nigam SN, Chandra S, Rupa Sridevi K, Manoha Bhukta A, Reddy GS, Nageswara Rao RC, Wright GC, Reddy PV, Deshmukh MP, Mathur RK, Basu MS, Vasundhara S, Vindhiya Varman P, Nagda AK (2005) Efficiency of physiological trait-based and empirical selection approaches for drought tolerance in groundnut. Ann App Biol 146: 433–439.

Ober ES, Bloa ML, Clark CJA, Royal A, Jaggard KW, Pidgeon JD (2005) Evaluation of physiological traits as indirect selection criteria for drought tolerance in sugar beet. Field Crops Res 91: 231–249.

Passioura JB (2002) Environmental biology and crop improvement. Funct Plant Biol 29: 537–546.

Paungbut D, Jogloy S, Kesmala T, Vorasoot N, Akkasaeng C, Patanothai A, Puppala N, (2011) Heritability of early season drought resistance traits and genotypic correlation of early season drought resistance and agronomic traits in peanut. SABRAO J Breed Genet 43(2): 165–187.

Reddy TY, Reddy VR, Anbumozhi V (2003) Physiological responses of peanut (*Arachis hypogaea* L.) to drought stress and its amelioration: a critical review. Plant Growth Regul 41: 75–88.

Reynolds M, Dreccer F, Trethowan R (2007) Drought-adaptive traits derived from wheat wild relatives and landraces. J Exp Bot 58: 177-186

Ricciardi A (1989) Plant breeding for resistance to drought. II. Relationships between stomatal and agronomic traits in *Vicia faba* L. genotypes. Agric Mediterr 119: 424–434.

Sekiya N, Yano K (2008) Stomatal density of cowpea correlates with carbon isotope discrimination in different phosphorus, water and $CO_2$ environments, New Phytol 179: 799–807.

Sheshshayee MS, Bindumadhava H, Rachaputi NR, Prasad TG, Udayakumar M, Wright GC, Nigam SN (2006) Leaf chlorophyll concentration relates to transpiration efficiency in peanut. Ann Appl Biol 148: 7–15.

Singh S, Russell MB (1981) Water use by maize/pigeonpeas intercrop on a deep Vertisol. In: Proceeding of International Workshop on Pigeonpeas, vol. 1. 15–19 December 1980, ICRISAT Center Patancheru, Andhra Pradesh, India, pp. 271–282.

Songsri P, Jogloy S, Vorasoot N, Akkasaeng C, Patanothai A, Holbrook CC (2008a) Root distribution of drought-resistant peanut genotypes in response to drought. J Agron Crop Sci 19: 92–103.

Songsri P, Jogloy S, Kesmala T, Vorasoot N, Akkasaeng C, Patanothai A, Holbrook CC, (2008b) Responses of reproductive characters of drought resistant peanut genotypes to drought. Asian J Plant Sci 7(5): 427–439.

Songsri P, Vorasoot N, Jogloy S, Kesmala T, Akkasaeng C, Patanothai A, Holbrook CC, (2009a) Evaluation of yield and reproductive efficiency in peanut (*Arachis hypogaea* L.) under different available soil water. Asian J Plant Sci 8(7): 465–473.

Songsri P, Jogloy S, Holbrook CC, Kesmala T, Vorasoot N, Akkasaeng C, Patanothai A, (2009b) Association of root, specific leaf area and SPAD chlorophyll meter reading to water use efficiency of peanut under different available soil water. Agric Water Manage 96: 790–798.

Soukup A, Malá J, Hrubcová M, Kálal J, Votrubová J, Cvikrová M (2004) Differences in anatomical structure and lignin content of roots of pedunculate oak and wild cherry-tree plantlets during acclimation. Biol Plant 48: 481–489.

Taiz L, Zeiger E (2006) Stress physiology, In: Taiz L, Zeiger E (Eds), Plant Physiology. Sinauer Associates, Inc., Sunderland, MA, pp. 671–681.

Tolk JA, Howell TA (2003) Water use efficiencies of grain sorghum grown in three USA southern Great Plains soils. Agric Water Manage 59: 97–111.

Van de Water PK, Leavitt SW, Betancourt JL (1994) Trends in stomatal density and 13C/ l2C ratios of *Pinus flexilis* needles during last glacial-interglacial cycle. Sci 264: 239–243.

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

Vorasoot N, Akkasaeng C, Songsri P, Jogloy S, Patanothai A (2004) Effect of available soil water on leaf development and dry matter partitioning in 4 cultivars of peanut (*Arachis hypogaea* L.). Songklanakarin J Sci Technol 26(6): 787–794.

Wright GC, Nageswara Rao RC (1994) Peanut water relations, In: Smartt J (Ed), The Peanut Crop. Chapman and Hall, London, UK, pp 281–325.

Zhang JW, Feng Z, Cregg BM, Schumann CM (1997) Carbon isotopic composition, gas exchange, and growth of three populations of ponderosa pine differing in drought tolerance. Tree Physiol 17: 461–466.