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Change of arginine content and some physiological traits under midseason drought in peanut genotypes with different levels of drought resistance

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Abstract: Peanut production areas frequently suffer from drought, which can cause severe yield losses, increased aflatoxin, and compositional changes in seed. Midseason drought is generally the most detrimental to seed yields and in altering seed protein composition. The purpose of this study was to investigate the effect of midseason drought on arginine content in peanut genotypes with different levels of drought resistance. The experiment was conducted under field conditions for 2 years. Two water regimes (well-watered conditions and no irrigation during 30–60 days after planting) were assigned as main plots, and five peanut genotypes were arranged in subplots. Arginine content of mature peanut seed was analyzed at harvest. Midseason drought increased arginine content in all genotypes in both years. Variation in arginine content among peanut genotypes also indicated the possibility for breeding programs to improve arginine content in peanut.

Key words: Amino acids, drought-sensitive, drought-tolerant, flowering stage, water deficit

1. Introduction

Peanut (*Arachis hypogaea* L.) is an important legume crop and a source of useful proteins, fatty acids, and the amino acid arginine. Human metabolism of arginine has been shown to have important roles in pathophysiology and cardiovascular physiology, largely via nitric oxide (NO)-dependent processes (Morris, 2005), and thus it plays important roles in many human health systems such as the cardiovascular, digestive, excretory, immune, metabolic, musculoskeletal, nervous, and reproductive systems (Elwardt, 2005). Peanut is therefore considered a food that contributes to a healthy diet.

Despite the high demand for peanut in Thailand, peanut production is insufficient to meet the country's needs. One of the major constraints to peanut production in Thailand is drought. Peanut is usually grown in arid and semiarid areas where the soil is very high in sand content and low in moisture holding capacity, and where rainfall is unpredictable. While drought can cause physiological changes at any growth stage, midseason drought is often the most detrimental in terms of yield loss. Past research has shown that midseason drought may cause changes in root systems (Jongrungklang et al., 2011), reductions of nodule dry weight and fixed nitrogen (Dinh et al., 2014), and changes in eicosenoic acid content (Dwivedi et al., 1996).

In general, drought causes an increase in amino acids in plants (Rai, 2002). Arginine is thought to help protect plants during drought conditions, increasing in rice leaves (Yang et al., 2000), *Brassica napus* leaves (Good and Zaplachinski, 1994), and Bermuda grass (Barnett and Naylor, 1966) during drought. Increases in free amino acids during drought, including arginine and proline, are thought to aid osmotic adjustment in peanut (Saini and Srivastava, 1981; Ali-Ahmad and Basha, 1998) and clover (Iannucci et al., 2002).

However, increased concentrations of arginine in the leaves due to drought conditions may not result in higher seed concentrations. In durum wheat, the highest concentration of arginine in the seeds occurred in an irrigated treatment, and arginine production was moderate under mild drought and lowest under more severe stress (Moral et al., 2007). In mature seeds of peanut, water deficit increased amino acids in 21 genotypes, but reduced

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concentrations were noted in 19 other genotypes (Jharna et al., 2013).

Genetic and environmental conditions are known to affect amino acid content in many plant species. Choudhary et al. (2005) found increased proline content in drought-tolerant rice leaves when compared to drought-sensitive genotypes. Kovács et al. (2012) noted a similar pattern in amino acid content in drought-tolerant wheat leaves. The objective of this study was to investigate the effect of midseason drought on arginine content in peanut seeds using genotypes that differ in their levels of resistance to drought. The results will be used in breeding programs targeting increased arginine content in peanut.

2. Material and methods

2.1. Location, duration, and experimental design

The experiment was conducted under field conditions for 2 years (November 2011 to March 2012 and November 2012 to March 2013) at the Field Crop Research Station of Khon Kaen University in Thailand. A split-plot design with four replications was used, with two water treatments assigned as the main plots (field capacity: FC; no irrigation during 30–60 days after planting: midseason drought, MD). Subplots were five peanut genotypes known to differ in pod yield under midseason drought (Jongrangklang et al., 2012).

The five genotypes selected were KKU 60 (a drought-tolerant cultivar with high root length density in the lower soil layer), ICGV 98305 (a drought-tolerant variety from the International Crops Research Institute for the Semi-Arid Tropics), Tifton 8 (a high-yielding drought-tolerant variety with a large root system), and KS 2 and Tainan 9 (both drought-susceptible genotypes with low pod yields under midseason drought) (Jongrungklang et al., 2012).

2.2. Crop management

Plot size was 5.5 × 5 m with a spacing of 50 cm between rows and 20 cm between plants within the row. Before planting, the seeds were treated with captan (3a,4,7,7a-tetrahydro-2-[(trichloromethyl) thio]-1H-isoindole-1,3 (2H)-dione) at the rate of 5 g kg^{-1} seeds to control *Aspergillus niger*. Three seeds were planted per hill. Alachor was sprayed for preemergent weed control at planting. *Rhizobium* (mixture of strains THA 201 and THA 205; Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand) was applied to peanut rows after planting. At 14 days after planting (DAP), seedlings were thinned to obtain 1 plant per hill. Gypsum was applied at the rate of 312.5 kg ha^{-1} at 28 DAP to supply calcium for pod development.

2.3. Irrigation

Drip irrigation was used for this study. On the planting date, water was supplied to each subplot at FC at the depth of 0–60 cm. Soil moisture content was maintained at FC to harvest for the nonstressed treatment. For the stress treatment (MD), irrigation was stopped during 30–60 DAP and resumed to FC at 60 DAP again until harvest. Irrigation needs were calculated as the sum of crop water requirements and soil evaporation (Sing and Russel, 1981; Doorenbos and Pruitt, 1992; Dinh et al., 2014) and applied daily to the appropriate plots.

2.4. Climate data, soil moisture content, and soil properties

Climate data including solar radiation (MJ m^{-2} day^{-1}), rainfall (mm), pan evaporation (mm), relative humidity (%), and minimum and maximum air temperature were recorded daily from planting to harvest using a nearby weather station. Soil moisture content was measured weekly from planting to harvest using a neutron probe (Type I.H. II SER, No. N0152, Ambe Diccot Instruments Co. Ltd., UK) at soil depths of 30, 60, and 90 cm in each subplot.

The soil type was a Yasothon series for both years with sand, silt, and clay of 93.9%, 4.7%, and 1.5% in the first year and 87.3%, 9.3%, and 3.4% in the second year. Soil pH, organic matter, total N, and cation exchange capacity were 6.5, 0.5%, 0.0%, and 4.2 cmol/kg respectively in the first year and 6.9, 0.6%, 0.0%, and 4.8 cmol/kg respectively in the second. Soil moisture content at the permanent wilting point was 4.8% and soil moisture content of FC was 10.9%.

2.5. Leaf relative water content (RWC)

Leaflets of five plants, collected from the second fully expanded leaf from the top of the main stem, were measured for RWC at 30, 45, 60, 75, and 90 DAP. Leaf samples were stored in sealed plastic bags and placed into a cooler for transport to the laboratory. Fresh weights were recorded within 2 h of the sample being taken, and samples were then soaked in distilled water under dim light at 25 °C for 8 h, after which saturated leaf weights were recorded. The samples were then oven-dried at 80 °C for at least 48 h to a constant weight when leaf dry weight was recorded. RWC was calculated based on the method of Gonzalez and Gonzalez-Vilar (2001).

2.6. Arginine content

Arginine content in seeds was measured at harvest. Only mature seeds were used for this study because arginine content is known to vary by seed maturity (Young et al., 1974). After pods were dried they were hand-shelled and sorted into maturity groups for arginine analysis. Seeds (150 g) were ground in a blender and subsamples of 10 g were further ground using a mortar and pestle. Samples were extracted with 30 mL of distilled water and filtered through No. 1 Whatman papers. Filtrate (5 mL) was used for determining arginine content. NaCl (6 M) was added to the filtrate and heated at 90 ± 2 °C for 90 min. The samples

were then centrifuged for 10 min at 5000 rpm and 1 mL of the supernatant was collected, transferred to a microtube, and set aside at 4 °C until analysis. Free arginine content was analyzed by the Sakaguchi test (Basha et al., 1976).

2.7. Stomatal conductance (SC), SPAD chlorophyll meter reading (SCMR), pod yield, and drought tolerance index (DTI)

SC was measured for 5 plants of each subplot between 1000 and 1200 hours using a Porometer-AP4 (Delta-T Devices, Cambridge, UK) at 60 DAP. SCMR was measured between 1000 and 1200 hours using 5 leaflets of each genotype at 60 DAP with a Minolta SPAD-502 meter.

Pod yield was recorded from 5 plants of each subplot at harvest. The pods were separated from the shoots. Pods were dried to obtain 8% moisture content and pod dry weight was determined. The DTI of each trait, including arginine content, was calculated by comparing drought stress measurements to those taken from well-watered plants as suggested by Girdthai et al. (2010).

2.8. Statistical analysis

Data were analyzed as a split-plot design using Statistix 8 (USDA NRCS, 2003). The data of 2 years were tested for homogeneity using the F-test (Gomez and Gomez, 1984). Arginine content was reported by year because of significant genotype × environment interactions. Mean comparisons were done using the least significant difference (LSD) (Gomez and Gomez, 1984).

3. Results

Years were slightly different for maximum temperatures and minimum temperatures (Figure 1). Solar radiation was 20.1 and 20.4 MJ m^{-2} day^{-1} in 2011/12 and 2012/13, respectively. Values of relative humidity ranged from 61% to 92% in 2011/12 and 55% to 92% in 2012/13, and

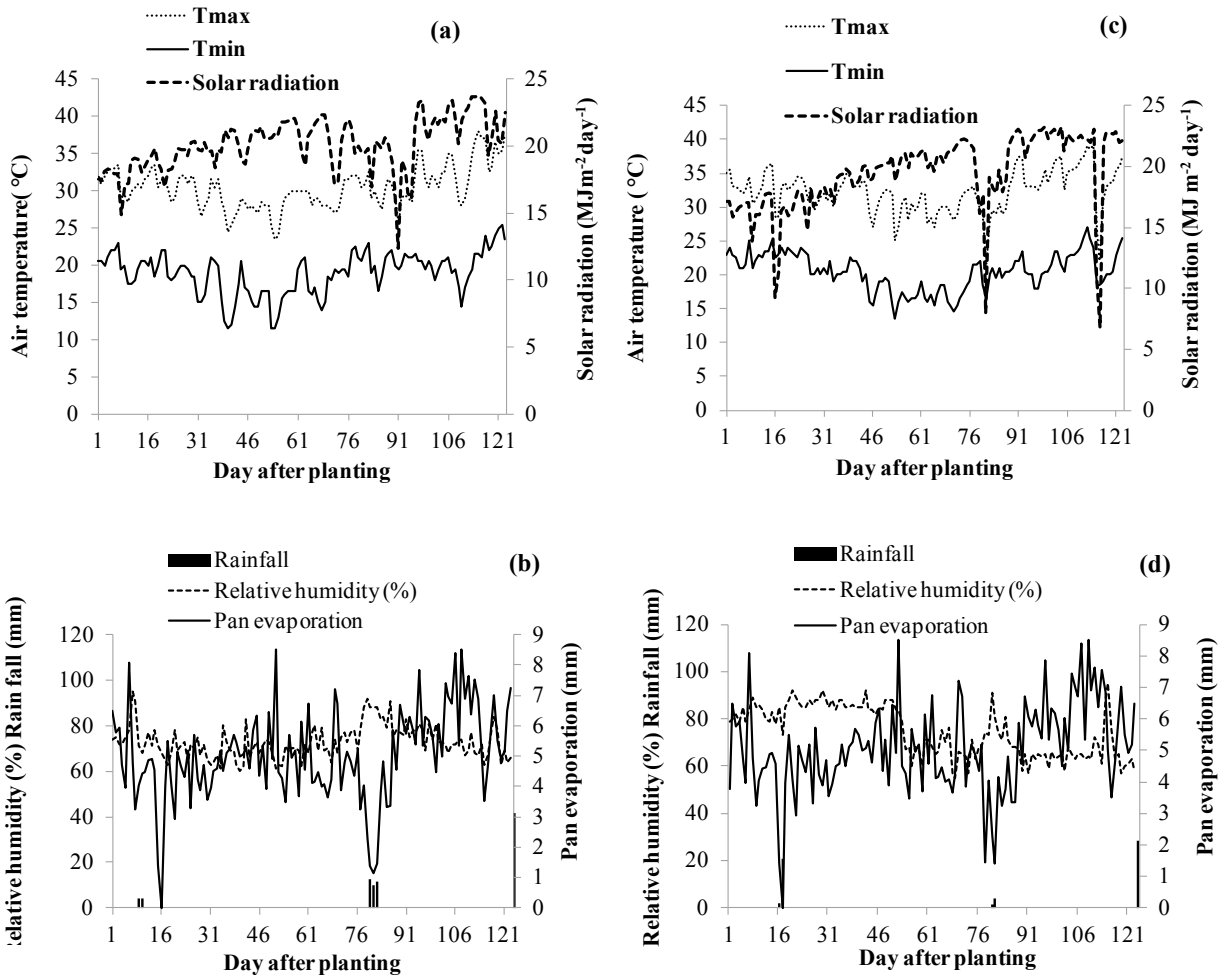

Figure 1. Minimum (Tmin) and maximum (Tmax) temperature, solar radiation, rainfall, evaporation, and relative humidity during November–March 2011/12 (year 1; a and b) and 2012/13 (year 2; c and d) in the field experiment, Khon Kaen University, Thailand.

rainfall totaled 41.8 and 28.5 mm in 2011/12 and 2012/13, respectively. Rainfall did not occur during the drought period in both years. Pan evaporations were similar between years (5.02 mm in 2011/12 and 5.03 mm in 2012/13). During water stress (30–60 DAP), the average values of all parameters were only slightly different between years, except for relative humidity. Relative humidity was 81.1% in the first year and 68.1% in the second year. The lower relative humidity in the second year resulted in more severe drought stress.

Water regimes were significantly different for soil moisture content at 30 cm of soil depth in both years. Soil moisture content was slightly different at a soil depth of 60 cm, and they were not different at 90 cm (Figure 2). Noticeably, from the graph of soil moisture content at the soil depth of 30 cm, plants in the second year were subjected to drought stress for a longer time than in the first year.

3.1. Combined analysis of variance

Years were significantly different for RWC in all periods ($P \leq 0.05$ and $P \leq 0.01$) (Table). Water treatments were significantly different for RWC ($P \leq 0.01$) at 60 DAP and genotypes were significantly different for RWC ($P \leq 0.05$ and $P \leq 0.01$) at 30, 60, and 90 DAP. The interaction between year and water treatment (Y × W) was significant for RWC at 60 DAP. Interaction between year and

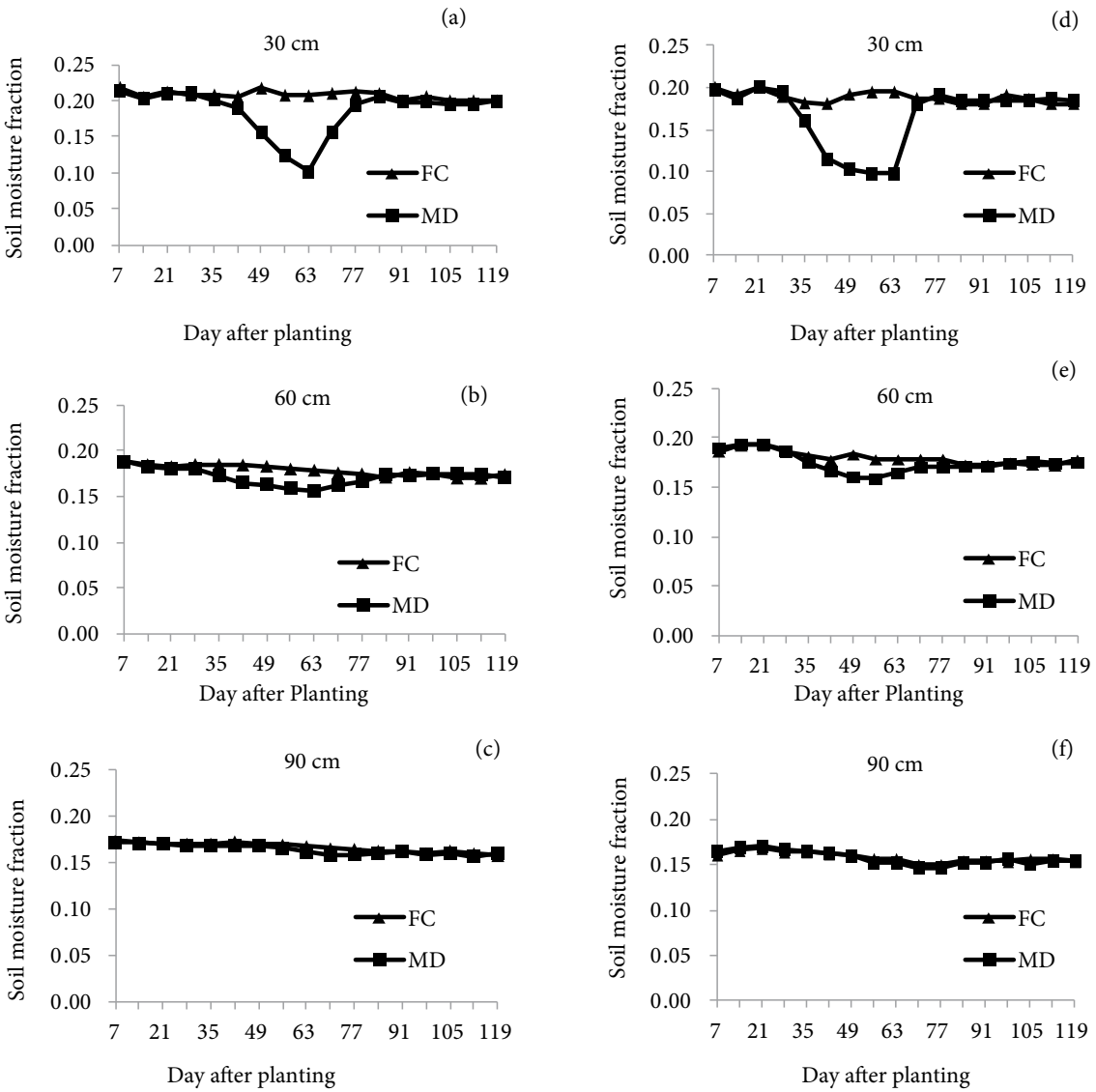

Figure 2. Volumetric soil moisture (fraction) in two water regimes as well-watered (FC) and midseason drought (MD) experiments conducted during November to March 2011/12 at 30 cm (a), 60 cm (b), and 90 cm (c) of the soil level and repeated during November to March 2012/13 at 30 cm (d), 60 cm (e), and 90 cm (f) of the soil level.

Table. Mean squares for relative water content (RWC) at 30, 60, and 90 days after planting (DAP) and arginine content of five peanut genotypes grown under field capacity (FC) and midseason drought (MD) in the dry seasons of 2011/12 and 2012/13.

Source	df	RWC			Arginine content
		30 DAP	60 DAP	90 DAP	
Year (Y)	1	4.65*	822.40**	162.08**	8376*
Rep. within Y	6	3.48	156.20	21.35	3853
Water regime (W)	1	0.25	1122.75**	8.57	51,788**
Y × W	1	0.63	518.67**	5.29	1
Error (a)	6	25.16	166.60	8.93	1523
Genotypes (G)	4	7.51*	209.47**	12.90*	57,212**
Y × G	4	4.84	103.42	10.69*	8786**
W × G	4	4.23	137.12*	2.74	1859*
Y × W × G	48	0.53	107.18	1.28	2217*
Error (b)	79	30.76	536.58	48.22	7374
CV (a) %		2.12	5.69	11.27	6.96
CV (b) %		0.83	3.61	1.04	5.41

df: degrees of freedom; * $P \leq 0.05$, ** $P \leq 0.01$.

genotype (Y × G) was significant for RWC at 90 DAP and interaction between year, water regime, and genotype (Y × W × G) was not significant. This indicated that years and genotypes were sources of variation for RWC in all periods and water treatment was a source of variation for RWC during the water stress period.

Years, water treatments, and genotypes were significantly different ($P \leq 0.05$ and $P \leq 0.01$) for arginine content (Table). Interaction between year and water treatment (Y × W) was not significant, whereas interaction between year, water treatment, and genotype (Y × W × G) was significant. Thus, years, water treatments, and genotypes were a main source of arginine variation.

3.2. Relative water content

RWC was not different between the two water treatments at 30 DAP (before the drought treatment began) in both years (Figure 3). At 45 and 60 DAP (15 and 30 days into the drought, respectively), RWC was significantly different in the second year, and the RWC at 60 DAP was different in the first year. After rewatering, the RWC of the two water treatments were similar for both 75 DAP and 90 DAP in both years.

3.3. Arginine content

Arginine content of peanut seeds was increased under midseason drought for all genotypes in both years (Figure 4). However, the magnitude of increase varied among peanut genotypes. In the first year, ICGV 98305 had the highest increase (44.1%) of arginine content under drought stress compared to the well-watered treatment. However, this genotype was lowest for arginine production when well-watered. Tifton 8 had the lowest increase (11.1%) for arginine content when subjected to drought stress.

3.4. Relationships between DTI (arginine) and DTI (RWC), DTI (SC), DTI (SCMR), and DTI (pod yield)

The correlations between the DTI for arginine and RWC were not significant in either year. The DTI for arginine was not correlated with the DTI for SC, SCMR, or pod yield in either year (Figure 5).

4. Discussion

In this study, arginine content was increased under midseason drought in all peanut genotypes for both years. Our results support previous findings that arginine content is increased under water deficit conditions. In rice leaves of 12-day-old seedlings, arginine content was increased at 4 h after stress and the highest production was observed at 12 h after stress (Yang et al., 2000). Similarly, in leaves of *Brassica napus*, most amino acids including arginine were increased under drought stress (Good and Zaplachinski, 1994); similar results were found in cotton (Parida et al., 2007). In peanut seedlings, drought stress increased the amount of total amino acids during drought periods and the amount decreased within 3 days after stress was relieved (Saini and Srivastava, 1981). The increase of arginine under drought stress is likely because it is a precursor of proline synthesis (Winter et al., 2015), and proline is an

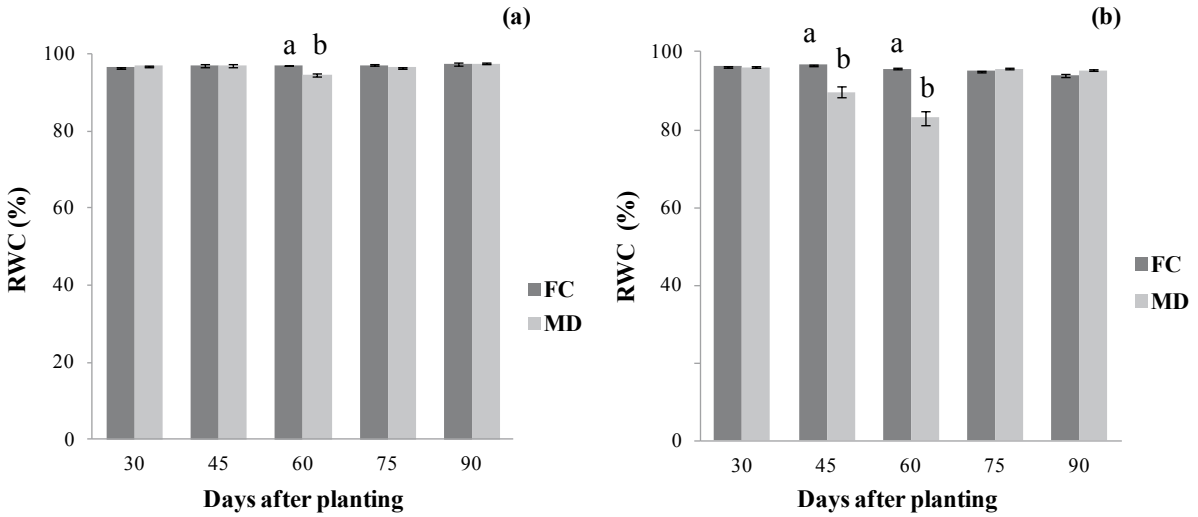

Figure 3. Leaf relative water content (RWC) of five peanut genotypes at 30, 45, 60, 75, and 90 days after planting in the dry season of 2011/2012 (year 1; a) and 2012/2013 (year 2; b); FC = field capacity and MD = midseason drought. Different letters indicate a significant difference ($P \leq 0.05$, LSD test, n = 20) between water regimes of each growth stage.

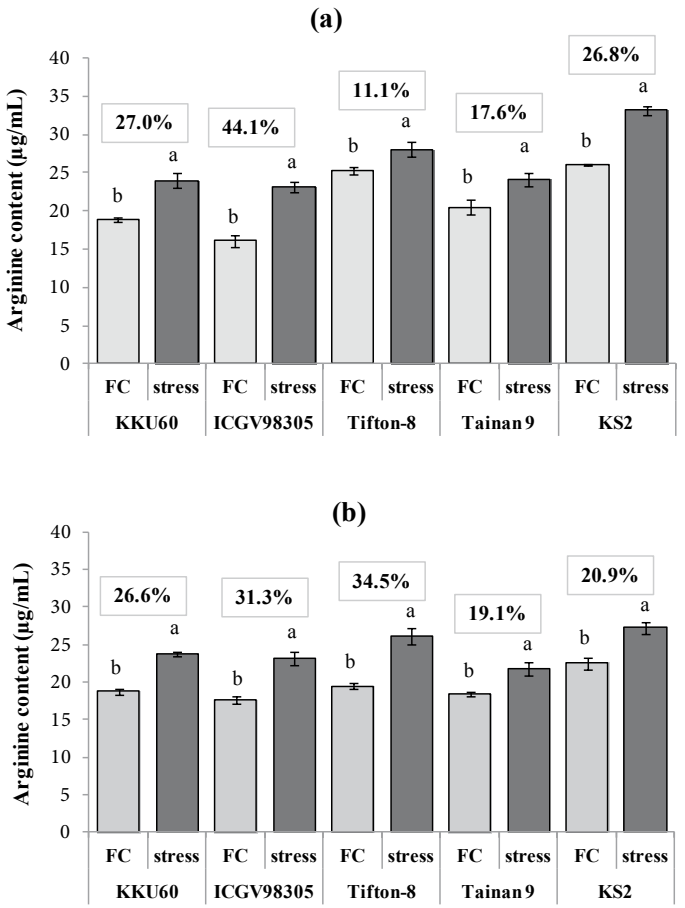

Figure 4. Arginine content (µg/g) in seeds of five peanut genotypes in the dry season of 2011/2012 (season 1; a) and 2012/2013 (season 2; b); FC = field capacity, stress = midseason drought. Numbers above bars are the percent increases of arginine production under drought stress compared to the well-watered treatment. Different letters indicate a significant difference ($P \leq 0.05$, LSD test, n = 4) between water regimes of each genotype.

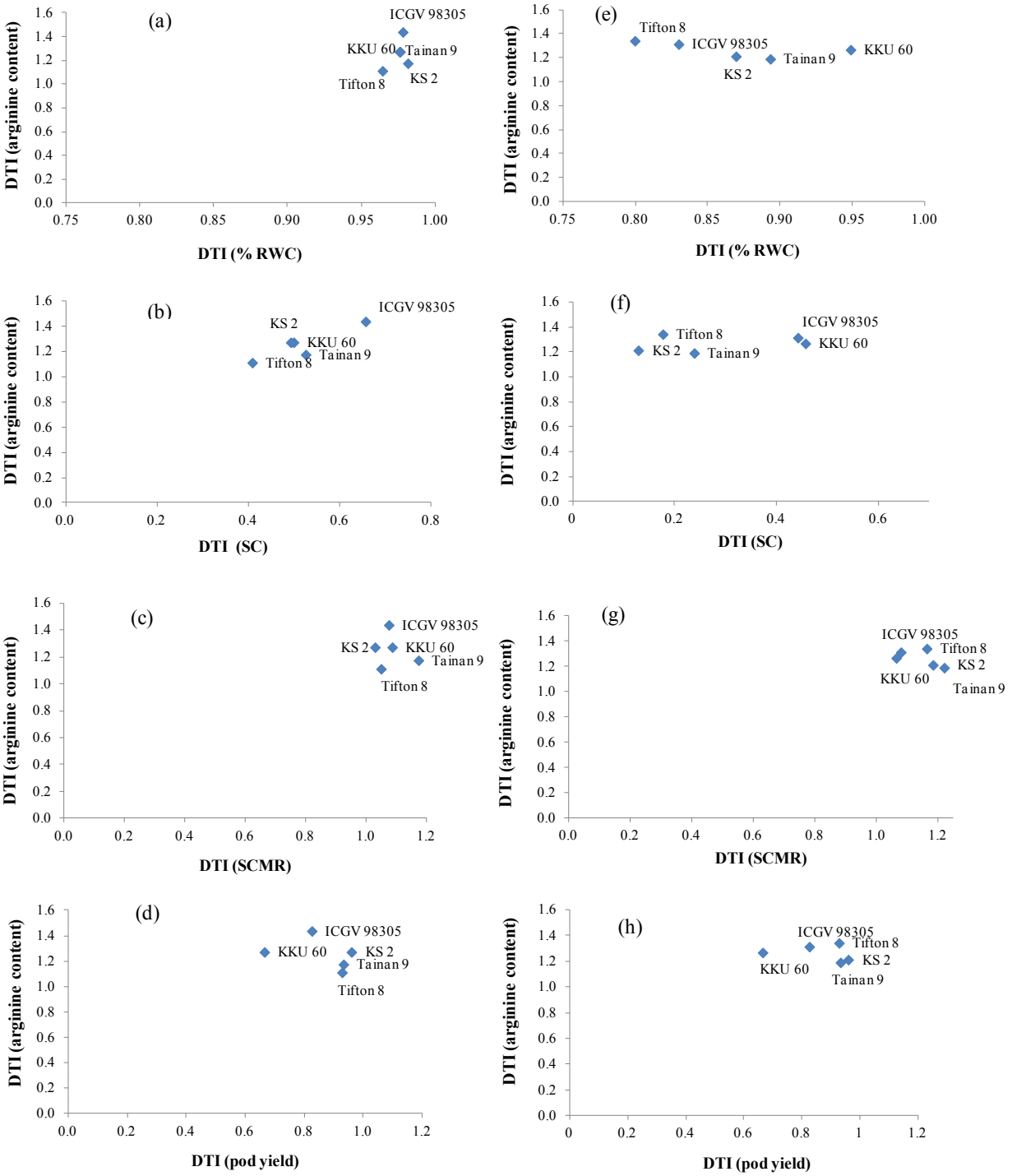

Figure 5. Relationships between drought tolerance index (DTI) of arginine and DTI of relative water content (RWC), DTI of stomatal conductance (SC), DTI of SPAD chlorophyll meter reading (SCMR), and DTI of pod yield of five peanut genotypes in 2011/12 (a, b, c, and d) and 2012/13 (e, f, g, and h).

important amino acid for osmotic adjustment in plants under drought stress (Alcázar et al., 2006).

However, drought stress does not always result in increased amino acid contents. Moral et al. (2007) reported that the amino acid content of durum wheat grown under drought varied according to genotype, with most genotypes showing a decrease in most amino acids, including arginine. In a study of 40 peanut genotypes by

Jharna et al. (2013), drought stress increased total amino acid content in 21 genotypes but reduced it in 19 genotypes.

In our study, midseason drought resulted in increased arginine accumulation in seeds at harvest. This increase in arginine may have been due to increased arginine synthesis or due to protein breakdown in the seeds under drought stress. Arginine is a precursor for the synthesis of proline and polyamines, which are important in protecting plants from drought stress (Minocha et al., 2014; Liu et al., 2015).

In our study, KS 2 had the highest arginine content under well-watered and stress conditions in both years. The arginine contents of KS 2 in 2011/12 were 26.1 and 33.1 µg/g for FC and MD conditions, respectively. The arginine contents of KS 2 in 2012/13 were 22.5 and 27.2 µg/g for FC and MD conditions, respectively. ICGV 98305 had the lowest arginine content under well-watered conditions in both years. However, it had higher arginine production under drought stress in both years (Figure 4; a 44.1% increase in 2011/12 and a 31.25 % increase in 2012/13). Tainan 9 had a low percentage of arginine production under drought stress in both 2011/12 and 2012/13, whereas Tifton 8 showed an 11.1% increase in 2011/12 and a 34.4% increase in 2012/13 due to drought. Thus, our results support previous findings that amino acid contents of peanut seed, when grown under drought stress, will vary with genotypes (Jharna et al., 2013).

In addition, our study found that arginine content was not a good predictor of drought resistance in peanut. This is comparable to previous studies by Parida et al. (2007), who found that proline and total free amino acids in cotton leaves increased under drought stress in both tolerant and susceptible genotypes, although the amount was higher in moderately tolerant genotypes than in susceptible genotypes. Similar results were also reported by Choudhary et al. (2005) for rice leaves monitored during drought. In contrast, Silvente et al. (2012) reported that alanine and glutamine decreased under drought stress in both tolerant and sensitive soybeans. Like Hanson (1982) and Basha (1992), we think that the differences in the results among different studies may be due to differences in plant species, genotypes, plant parts, seasons, and the duration of drought

In our study, the increase in arginine under drought stress was not related to any of the physiological traits that we measured (RWC, SC, SCMR, and pod yield). The results of our study, like others, indicate that the increase of arginine content is likely due to the plant responding to drought by increasing osmotic potential in various plant parts. Our results also indicate that selection for high arginine content, beneficial physiological traits, and high yield is possible.

In conclusion, drought from the flowering stage to seed setting (30–60 DAP) increased arginine content in peanut seeds and the amounts varied between years and among peanut genotypes. Based on this study, breeding of peanut for high arginine content and drought tolerance should be possible.

Acknowledgments

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References

Alcázar R, Marco F, Cuevas JC, Patron M, Ferrando A, Carrasco P, Tiburcio AF, Altabella T (2006). Involvement of polyamines in plant response to abiotic stress. Biotechnol Lett 28: 1867-1876.

Ali-Ahmad M, Basha SM (1998). Effect of water stress on composition of peanut leaves. Peanut Sci 25: 31-34.

Barnett NM, Naylor AW (1966). Amino acid and protein metabolism in Bermuda grass during water stress. Plant Physiol 41: 1222-1230.

Basha SM (1992). Effect of location and season on peanut seed protein and polypeptide composition. J Agri Food Chem 40: 1784-1788.

Basha SM, Cherry JP, Young CT (1976). Change in free amino acids, carbohydrates, and protein of maturing seeds from various peanut (*Arachis hypogaea* L.) cultivars. Cereal Chem 53: 586-597.

Choudhary NL, Sairam RK, Tyagi A (2005). Expression of Δ^1-pyrroline-5-carboxylate synthetase gene during drought in rice (*Oryza sativa* L.). Indian J Biochem Biophys 42: 366-370.

Dinh HT, Kaewpradit W, Jogloy S, Vorasoot N, Patanothai A (2014). Nutrient uptake of peanut genotypes with different levels of drought tolerance under midseason drought. Turk J Agric For 38: 495-505.

Doorenbos J, Pruitt WO (1992). Crop Water Requirements. FAO Irrigation and Drainage Paper 24. Rome, Italy: FAO.

Dwivedi SL, Nigam S, Nageswara Rao NRC, Singh U, Rao KVS (1996). Effect of drought on oil, fatty acid and protein content of groundnut (*Arachis hypogaea* L.) seeds. Field Crop Res 48: 125-133.

Elwardt H (2005). Health Benefits of Arginine. Hove, UK: Arkworld International.

Fukutoku Y, Yamada Y (198). Diurnal changes in water potential and free amino acid contents of water-stressed and non-stressed soybean plants. Soil Sci Plant Nutr 27: 195-204.

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.

Liu JH, Wang W, Wu H, Gong X, Moriguchi T (2015). Polyamines function in stress tolerance: from synthesis to regulation. Front Plant Sci 6: 827.

Minocha R, Majumdar R, Minocha SC (2014). Polyamines and abiotic stress in plants: a complex relationship. Front Plant Sci 5: 175.

Moral LF, Rharrabti Y, Martos V, Royo C (2007). Environmentally induced changes in amino acid composition in the grain of durum wheat grown under different water and temperature regimes in a Mediterranean environment. J Agri Food Chem 5: 8144-8151.

Gomez KA, Gomez AA (1984). Statistical Procedures for Agricultural Research. 2nd ed. New York, NY, USA: John Wiley & Sons.

Gonzalez L, Gonzalez-Vilar M (2001). Determination of relative water content. In: Reigosa Roger MJ, editor. Handbook of Plant Ecophysiology Techniques. Berlin, Germany: Springer, pp. 207-212.

Good AG, Zaplachinski ST (1994). The effects of drought stress on free amino acid accumulation and protein synthesis in *Brassica napus*. Physiol Plant 90: 9-14.

Hanson AD (1982). Metabolic responses of mesophytes to plant water deficits. Annu Rev Plant Physiol 33: 163-203.

Jharna DE, Chowdhury BLD, Rana MAM, Sharmin S (2013). Selection of drought tolerant groundnut genotypes (*Arachis hypogaea* L.) based on total sugar and free amino acid content. J Environ Sci Nat Resour 6: 1-5.

Jongrungklang N, Toomsan B, Vorasoot N, Jogloy S, Boote KJ, Hoogenboom G, Patanothai A (2011). Rooting traits of peanut genotypes with different yield responses to pre-flowering drought stress. Field Crop Res 120: 262-270.

Kovács Z, Simon-Sarkadi L, Vashegyi I, Kocsy G (2012). Different accumulation of free amino acids during short-and long-term osmotic stress in wheat. Scientific World Journal 2012: 216521.

Morris SM Jr (2005). Arginine metabolism in vascular biology and disease. Vas Med 10 (Suppl. 1): 83-87.

Parida AK, Dagaonkar VS, Phalak MS, Umalkar GV, Aurangabadkar LP (2007). Alterations in photosynthetic pigments, protein and osmotic components in cotton genotypes subjected to short-term drought stress followed by recovery. Plant Biotechnol Rep 1: 37-48.

Rai VK (2002). Role of amino acids in plant responses to stresses. Biol Plantarum 45: 481-487.

Saini HS, Srivastava AK (1981). Osmotic stress and the nitrogen metabolism of two groundnut (*Arachis hypogaea* L.) cultivars. Irrigation Sci 2: 185-192.

Silvente S, Sobolev AP, Lara M (2012). Metabolite adjustments in drought tolerant and sensitive soybean genotypes in response to water stress. PLoS One 7: e38554.

Singh S, Russel MB (1981). Water use by maize/pigeonpea intercrop on a deep Vertisol. In: Proceedings of the International Workshop on Pigeonpeas, 15–19 December 1980. Patancheru, India: ICRISAT Center, pp. 271-282.

USDA NRCS (2003). Statistix 8: Analytical Software User's Manual. Tallahassee, FL, USA: USDA.

Winter G, Todd CD, Trovato M, Forlani G, Funck D (2015). Physiological implications of arginine metabolism in plants. Front Plant Sci 6: 534.

Yang CW, Lin CC, Kao CH (2000). Proline, ornithine, arginine and glutamic acid contents in detached rice leaves. Biol Plantarum 43: 305-307.

Young CT, Matlock RS, Mason ME, Waller GR (1974). Effect of harvest date and maturity upon free amino acid levels in three varieties of peanuts. J Am Oil Chem Soc 51: 269-273.