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ARTICLE

Genotypic Differences in Photosynthetic Limitations to Carbon Assimilation in Peanut under Drought at the Onset of Flowering

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ABSTRACT

Drought can greatly limit carbon assimilation in plants. However, different species have distinct photosynthetic components governing limitations to photosynthesis exposed to drought conditions. Furthermore, intra-species variations in photosynthetic response to drought is also expected. Information on underlying limitations to carbon assimilation in peanut (*Arachis hypogaea* L.) has been controversial. Therefore, this study aimed to verify potential drought tolerance associated with the photosynthetic process within ten diverse peanut genotypes grown under drought as well as to determine the limitation to carbon assimilation in these genotypes and identify parameter(s) that can be used as a reference indicator of photosynthesis response to drought intensity. Experiments were conducted in 2017 and 2018 using rainout shelters to impose drought for 40 days starting 34 days after planting. Ten peanut genotypes were planted in two blocks, one fully irrigated and one under drought stress during reproductive development. Photosynthetic measurements were taken at 25 and 40 days after onset of stress. C76-16 was identified as the most tolerant genotype due to improved plasticity by downregulating photosynthesis under mild drought stress (25 progressive days under drought) and upregulating multiple photosynthetic component processes under more severe drought (40 days under drought) to sustain photosynthesis. The primary limitation to photosynthesis across all peanut genotypes was stomatal conductance, whereas non-stomatal factors (photochemical reactions) were nearly unaffected by mild drought. In addition, stomatal conductance and electron flux to CO_2 assimilation contributed most to drought tolerance in peanut genotypes. Moreover, these two photosynthetic component processes can be jointly used as reference indicators of photosynthetic status of peanut under varying drought intensities.

INTRODUCTION

Drought is one of the main environmental factors limiting photosynthesis in plants, thus leading to reduced growth and yield (Kramer, 1983). Stomatal closure is recognized as the primary response to drought conditions for a number of plant species (Cornic, 2000). The limiting component in the photosynthetic process of plants under water-limiting conditions has been extensively studied (Ennahli and Earl, 2005; Lauriano et al., 2004; Lawlor, 2002; Medrano et al., 2002). It is generally accepted that stomatal closure and increased mesophyll resistance, followed by a decrease in CO_2 concentration in the intercellular space and chloroplast stroma, are the underlying limiting factors to photosynthesis under mild to moderate drought stress (Flexas et al., 2006). The diffusion of CO_2 starts by entering open stomata, diffusing through intercellular air spaces, cell wall and cytosol, then through the chloroplast envelope until reaching the chloroplast stroma to be fixed by ribulose bisphosphate carboxylase (Rubisco) in the carboxylation site (Evans et al., 2009). In plants experiencing drought stress, stomatal closure prevents CO_2 from entering the leaves, eventually depleting CO_2 concentration in the intercellular spaces and the chloroplast stroma (Evans et al., 2009). Stomatal closure in response to drought conditions is regulated by the efflux of K^+ along with the influx of Ca^{+2} in the guard cells, triggered by abscisic acid produced in the roots (Luan 2002).

When drought becomes more severe, functionality of the thylakoid reactions along with Rubisco activity govern the reductions in photosynthesis (Flexas and Medrano, 2002). Severe drought reduces photochemical activity, decreasing quantum yield of Photosystem II (Φ_{PSII}) and adenosine triphosphate (ATP) synthesis, with consequent impairment of RuBP (ribulose-1,5-bisphosphate) regeneration, and photoinhibition due to an excess of light energy. The over-production of reactive oxygen species (ROS) that inactivate protein synthesis for PSII repair (Keren and Krieger-Liszkay, 2011) damages PSII structures in comparison with the repair rate. Alternatively, the photorespiratory pathway can serve as a sink for excess light energy. Energy partitioning to photorespiration can contribute to mitigation of photoinhibition by maintaining the metabolic process through the use of electron transport products (Nortor et al., 2002). However, this mechanism also generates ROS, increasing the risk for oxidative damage in photosynthetic cells.

Photosynthetic response to drought conditions is quite complex and involves multiple physiological and metabolic processes. Moreover, other factors such as duration and severity of drought, plant growth stage when drought is experienced, can directly influence a plant's response. There are also intra- and inter-species differences in the photosynthetic response to drought. For instance, Chastain et al. (2024) observed that compared to non-stomatal factors, stomatal limitation was the main mechanism contributing to decreased photosynthesis in multiple cotton genotypes (*Gossypium hirsutum* L. and *G. barbadense* L.) under moderate drought. Conversely, Pilon et al. (2018) reported non-stomatal components including electron transport through the PSII as primary limiting factors impairing photosynthesis in the peanut (*Arachis hypogaea* L.) cv. Georgia-06G (Branch 2007) grown under progressive drought stress. Lauriano et al. (2004) suggested both stomatal and non-stomatal limitations to photosynthetic rate in three peanut genotypes under water-deficit stress.

Different species and genotypes within a given species may possess distinct mechanisms to cope with drought conditions. Plant adaptations to drought include isohydric *versus* anisohydric strategies as well as phenotypic plasticity. Isohydric plants avoid drought by closing stomata and maintaining water potential to prevent xylem cavitation (water column breakage). The disadvantages of this strategy are the potential carbon starvation due to reduction in CO_2 diffusion through the leaf and the decrease in transpiration, leading to increased leaf temperature and causing cell damage. Conversely, anisohydric plants maintain stomatal opening and transpiration, sustaining photosynthesis. This strategy can reduce leaf water potential and increase the likelihood of hydraulic failure by xylem cavitation (McDowell et al., 2008).

Phenotypic plasticity is also known as an important mechanism for some species and/or genotypes to produce more than one response to a given environmental condition (West-Eberhard, 1989). Plasticity is generally associated with a specific trait or trait complex (Schlichting, 1986). For instance, one genotype that confers plasticity in the photosynthetic component processes under drought stress may not be plastic to light intensity changes or other stresses. Identifying phenotypic plasticity associated with photosynthetic efficiency in breeding lines or advanced genotypes is of immense relevance for crop production in the face of climate change.

Several authors documented drought-induced inhibition of photosynthetic components as well as primary mechanisms contributing to drought tolerance in peanut (Reddy et al., 2003; Kottapalli et al., 2009; Lauriano et al., 1997; Zhang et al., 2022). Reddy et al. (2003) suggested that reductions in net photosynthesis caused by drought were directly associated with stomatal conductance. Kottapalli et al. (2009) examined 20 genotypes from two peanut subspecies, *hypogaea* and *fastigiata*, for physiological tolerance to water-deficit stress. The authors identified a downregulation of photosynthesis by stomatal closure followed by a decrease in chlorophyll *a* fluorescence in an attempt to preserve water and prevent cellular damage. Conversely, a study on the photosynthetic capacity of three peanut cultivars, subspecies *hypogaea*, attributed declines in net photosynthesis (gross carbon assimilation minus respiratory losses) to non-stomatal factors, i.e. decreased activity of PSII and PSI electron flux (Lauriano et al., 1997). Zhang et al. (2022) screened 38 peanut genotypes and landraces for drought tolerance. Results indicated that genotypes differed in the mechanism to cope with drought and sustain photosynthesis. Some genotypes were classified as "water spenders" (i.e. anisohydric), maintaining stomata open, whereas other genotypes were defined as "water savers" (i.e. isohydric), closing stomata and reducing water consumption. Although the aforementioned studies greatly contribute to advancing knowledge on photosynthetic response of peanut plants to drought, none of them discuss collectively the underlying photosynthetic component processes (stomatal and non-stomatal) contributing to photosynthetic limitations along with phenotype plasticity as a drought tolerance mechanism in peanut genotypes. Therefore, the objectives of this study were to 1) verify potential drought tolerance associated with the photosynthetic process within ten diverse peanut genotypes, 2) identify the primary limitation to carbon assimilation in the

peanut genotypes under progressive drought during reproductive development, 3) define the photosynthetic parameter(s) with the greatest contribution to photosynthetic drought tolerance in peanut, and 4) determine if the given parameter(s) can be used as reference indicators of photosynthesis response to drought intensity.

MATERIALS AND METHODS

Study site, genotypes, and water treatments.

Experiments were conducted during the 2017 and 2018 seasons at the Gibbs Experimental Farm of the University of Georgia located in Tifton, GA (31°43'N, 83°58'W). Ten peanut genotypes were planted in two blocks, one fully irrigated and one under drought stress during reproductive development. Drought stress was imposed by withholding water from the drought block, allowing soil to dry progressively for 40 days. The list of genotypes along with characteristics from each genotype that served as selection criteria for this study is described in Table 1. Briefly, Georgia-06G is the cultivar most planted in Georgia and was included as a 'standard check'. Florida-07 has been previously reported as drought susceptible; therefore, it was included as a 'negative check' (Luis et al., 2016). Other genotypes were selected either due to their large root system and some resistance to aflatoxin (which is directly associated with late-season drought), or small root system for comparison of photosynthetic performance. Each genotype was replicated five times within each block. Plots were 1.5 m long and 1.8 m wide. Small plot size is common in experiments involving breeding lines/genotypes due to limited number of seeds. Seeds were sown on June 1, 2017 and June 8, 2018 at a 0.91 m inter-row spacing and seeding rate of 20 seeds m^{-1}. Soil was classified as Tifton sandy loam (fine loamy, kaolinitic, thermic Plinthic Kandiudults). The drought treatments were imposed on July 5, 2017 and July 12, 2018 by covering the plots with rainout shelters. The shelters were 24.4 x 9.1 m built with metal and covered with a single layer of clear poly covering on roof, sides and gables. Drought stress started 34 days after planting (DAP) at the onset of flowering and continued for a total of 40 days. Two sets of physiological measurements were taken during the drought stress period, 25 and 40 days after the onset of stress. First measurements were taken on July 30, 2017 and August 6, 2018 for the first drought period (25 days of stress or 59 days after planting) as well as on August 14, 2017 and August 21, 2018 for the second drought period (40 days of stress or 74 days after planting). After the second measurements were taken, rainout shelters were removed and all plots were irrigated to field capacity and then irrigated when needed until the end of the season. The fully irrigated control received irrigation as needed according to the University of Georgia Extension recommendations using rain gun sprinklers (Porter, 2022). All other management practices were performed following recommendations by the University of Georgia Extension given in Monfort et al. (2022).

Table 1. List of peanut genotypes and description of characteristics used as selection criteria for this study.

Genotype	Description	Reference
A72	Low resistance to aflatoxin production Moderate drought susceptibility	Luis et al. (2016); C. Holbrook (personal commun.)
A100	Small root system	C. Holbrook (personal commun.)
C75-13	Large root system	C. Holbrook (personal commun.)
C76-16	Drought tolerance Relative resistance to aflatoxin production	Luis et al. (2016); Dang et al. (2012); Pilon et al. (unpublished); C. Holbrook (personal commun.)
C431-1-1	Very large root system	C. Holbrook (personal commun.)
C431-1-4	Very large root system	C. Holbrook (personal commun.)
Florida-07	Drought susceptible	Luis et al. (2016)
GA-06G	Moderate drought tolerance Most planted cultivar in Georgia	Branch (2007); Branch and Fletcher (2017); Pilon et al. (2018)
Tifrunner	Moderate drought tolerance	Holbrook and Culbreath (2007); Zhang et al. (2022)
Tifton-8	Large root system Moderate drought tolerance	Rucker et al. (1995)

At the end of each drought stress period, survey measurements of gas exchange and fluorescence were obtained using the LI-6400 XT portable infrared gas analyser (LI-COR, Lincoln NE) coupled with a fluorometer chamber (Model LI-6400-40, LI-COR, Lincoln, NE). Measurements were taken on the uppermost, fully expanded leaf from the mainstem (node 2 from the terminal) between 1200 and 1400 h (±1 h solar noon). The environmental settings for the leaf chamber included photosynthetically active radiation = 1500 µmol $m^{-2}s^{-1}$, reference CO_2 concentration = 400 µmol mol^{-1}, flow rate = 500 µmol s^{-1}, relative humidity = 60 ± 10%, and air temperature = ambient temperature at time of measurement. Modulation light settings recommended for light-adjusted leaves were used to obtain steady state fluorescence (Fs), and the multi-phase flash

protocol was used to estimate maximum fluorescence intensity (Fm'). Net photosynthesis (A_N), stomatal conductance (g_{sw}), intercellular CO_2 concentration (C_i), quantum yield of photosystem II (Φ_{PSII}), and electron transport rate (ETR) were the parameters of interest obtained from the equipment. The additional parameters electron flux to carbon assimilation (ETR_a), electron flux to photorespiration (ETR_p), and CO_2 concentration in the chloroplast stroma (C_c) were derived according to equations given below.

$$ETR_a = 1/3\ (ETR + 8(A_N + R_D))$$

$$ETR_p = 2/3\ (ETR - 4(A_N + R_D))$$

$$C_c = ETR_a / ETR_p \times O / K_S$$

in which R_D is the respiration rate, O is the oxygen concentration at the carboxylation site, and Ks is the Rubisco specificity factor (Galmés et al., 2007; Snider et al., 2022).

Pod yield and quality were planned to be assessed at the end of the season in both years. However, the experiments were severely impacted by hurricanes Irma and Michael that occurred in September 2017 and October 2018, respectively (NOAA, 2017; 2018). Plants were damaged, compromising the results. Therefore, only the physiological assessment will be presented.

Statistical analysis.

The statistical model considered only the comparison among the genotypes within each water regime and stress timing. Comparisons between the two drought events were not performed as they would not address the objectives of this study. Genotype within each water regime block was considered a fixed effect and was nested within year. Replication and genotype × replication were considered as random effects. Data were subjected to a one-way analysis of variance and Tukey's Honestly Significant Difference at 5% probability was used as a post-hoc test for mean comparison. Relative values for the photosynthetic parameters were calculated using the average of five replicates as follows:

$$\text{Relative value} = (PV_{drought} / PV_{control}) - 1$$

in which PV indicates the value for the photosynthetic parameter of interest.

Pearson's correlation (p = 0.05) was used to assess the relationship between absolute values of net photosynthesis and multiple diffusional and photochemical parameters. Regression analysis was further used to identify the coefficient of determination of pairwise correlations. All statistical analyses were conducted using JMP Pro 17 (Cary, NC).

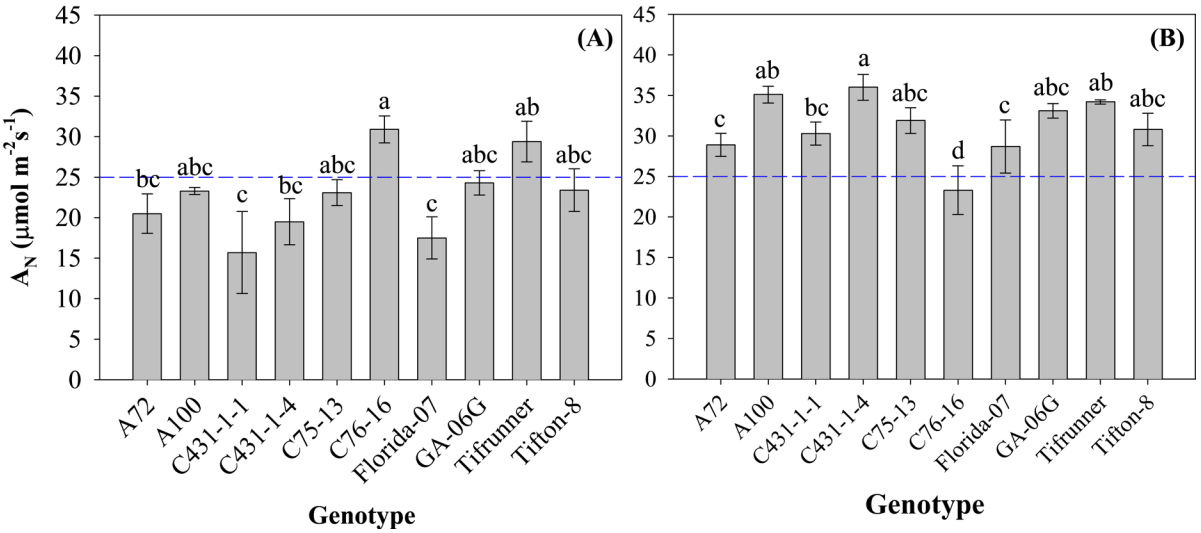

Figure 1. Net photosynthesis (µmol $m^{-2}s^{-1}$; A_N) in ten peanut genotypes grown under fully irrigated conditions measured at (A) 59 days after planting (DAP) and (B) 74 DAP. Blue dashed reference lines indicate a "lower threshold" for net photosynthesis in C3 plants. Values are means (n = 10) and bars not sharing a common letter denote significant differences among genotypes according to Tukey's Honestly Significant Difference test at 5% probability.

RESULTS AND DISCUSSION

Differences in net photosynthesis under irrigated conditions were observed among genotypes at 59 days after planting (DAP; Fig. 1A) and 74 DAP (Fig. 1B). At 59 DAP, C76-16 had 77, 58, and 51% greater net photosynthesis than Florida-07, C431-1-1, and A72, respectively, under irrigated conditions (Fig. 1A). Under optimal conditions (adequate soil moisture and air temperature of around 30 °C), net photosynthetic rates in peanut are generally between 30 and 35 µmol $m^{-2}s^{-1}$, and rates below 25 µmol $m^{-2}s^{-1}$ can indicate a decrease in efficiency to assimilate CO_2 and produce photoassimilates. C76-16 and Tifrunner were the only genotypes with average net photosynthesis greater than 25 µmol $m^{-2}s^{-1}$. Conversely, at 74 DAP, C76-16 had A_N lower than 25 µmol $m^{-2}s^{-1}$, which was the lowest rate across all genotypes (Fig. 1B). The photosynthetic process is functionally flexible, allowing for a dynamic mechanism rapidly coping with varying environmental conditions. In addition, plant growth stage along with seasonal variation and diurnal rhythm can lead to different photosynthetic responses (Eberhard et al., 2008).

Therefore, it is not uncommon to observe variations in A_N within a genotype over time. However, it is crucial to explore A_N response between water regimes to identify potential tolerance or plasticity within genotypes. When the peanut genotypes were exposed to progressive drought stress (25 and 40 days, starting at 34 DAP), no differences in A_N were observed among them on either measurement date (Fig. 2). Overall average A_N was 22.9 µmol $m^{-2}s^{-1}$ at 25 DAS (Fig. 2A) and 24.2 µmol $m^{-2}s^{-1}$ at 40 DAS (Fig. 2B). Due to variations in genotypic response under irrigated conditions and minimum variation under drought stress, the relative A_N values between two watering regimes was calculated to identify genotypes with greater tolerance to drought or increased plasticity. At 25 DAS, A72, A100, C431-1-1, Florida-07, and Tifton-8 were capable of increasing the photosynthetic rate under drought compared with irrigated conditions (Fig. 3). However, as drought progressed (40 days of stress), only C76-16 showed a positive value, with slightly greater A_N in stressed plants compared with those grown under fully irrigated conditions (Fig. 3). Genotypes with ability to respond to a stressing condition by maintaining or improving physiological processes, such as photosynthesis, likely possess improved phenotypic plasticity (West-Eberhard, 2003). The mechanisms of plasticity allow for phenotypic adjustments in plants in response to environmental conditions without genetic alterations. Although not within the scope of this study, it is noteworthy that plant acclimation to suboptimal environmental conditions, such as drought, heat, and light intensity, involves molecular-level adjustments by photosynthetic gene regulation through DNA methylation (Yaish, 2013). This study focuses on the actual photosynthetic components' response of varying peanut genotypes to progressive drought.

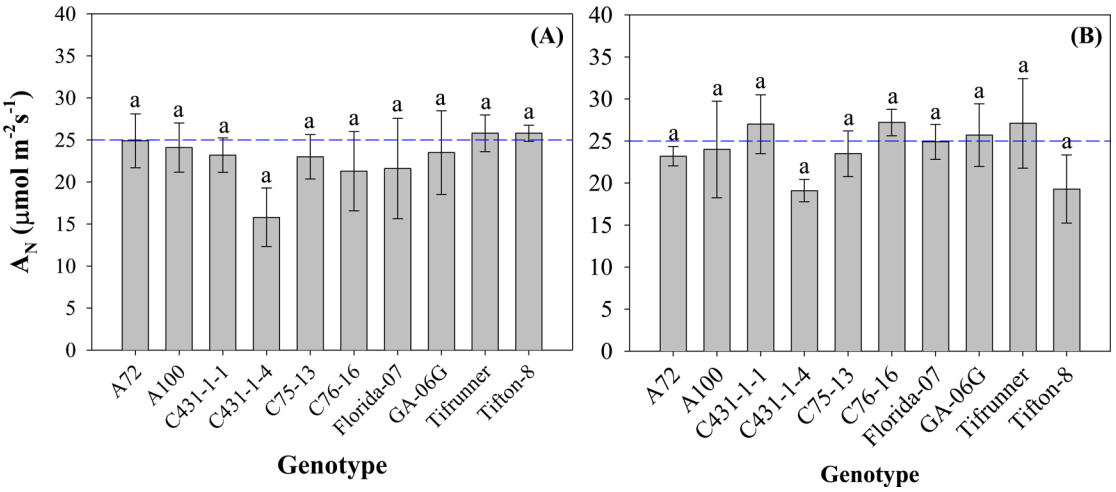

Figure 2. Net photosynthesis (µmol $m^{-2}s^{-1}$; A_N) in ten peanut genotypes grown under drought conditions for (A) 25 days and (B) 40 days, starting at 34 days after planting (or 59 and 74 days after planting, respectively). Blue dashed reference lines indicate "lower threshold" net photosynthesis in C3 plants. Values are means (n = 10) and bars not sharing a common letter denote significant differences among genotypes according to Tukey's Honestly Significant Difference test at 5% probability.

A decline in A_N can be associated with stomatal and non-stomatal limiting factors. Stomatal conductance is recognized as one of the primary responses of leaves to drought stress in several C3 species (Cornic, 2000). However, studies in peanut have also indicated that declines in photosynthesis were driven by an inhibition of the thylakoid reactions under progressive drought stress (Pilon et al., 2018; Lauriano et al., 2004). To identify the limiting factors to net photosynthesis in this range of genotypes, relative values were also derived for multiple photosynthetic (stomatal and non-stomatal) parameters. At 25 DAS, g_{sw} and C_c (CO_2 concentration in the chloroplast stroma) were the parameters with greater variation among the genotypes (Fig. 4). The positive values for g_{sw} ration for genotypes A72, C431-1-1, C75-13, Florida-07, and Tifton-8 in Fig. 4 suggest small reduction in stomatal closure of these genotypes in response to the drought treatment. The aforementioned genotypes and GA-06G also increased C_c. Intercellular CO_2 concentration (C_i) was decreased in all genotypes when plants were exposed to drought for 25 days (Fig. 4). High CO_2 concentration in the chloroplast stroma is important to sustain photosynthesis and prevent photodamage (Eberhard et al., 2008). Genotypes that can maintain high CO_2 levels in the chloroplast stroma decrease the affinity of ribulose 1,5-bisphosphate (RuBP) to oxygen, thus increasing the speed of RuBP carboxylation in the carbon fixation reaction (first step of Calvin cycle for the formation of glucose). When there is low CO_2 concentration in the chloroplast stroma, RuBP increases affinity with O_2 entering the photorespiratory pathway (Terashima et al., 2001). Photorespiration serves as an energy sink, consuming ATP and NADPH; however, it has a protective role under low stomatal conductance, preventing over-reduction of the plastoquinone pool in the electron transport chain of the thylakoid reactions (Huner et al., 2002). Our results did not show genotypic variations among genotypes for C_i.

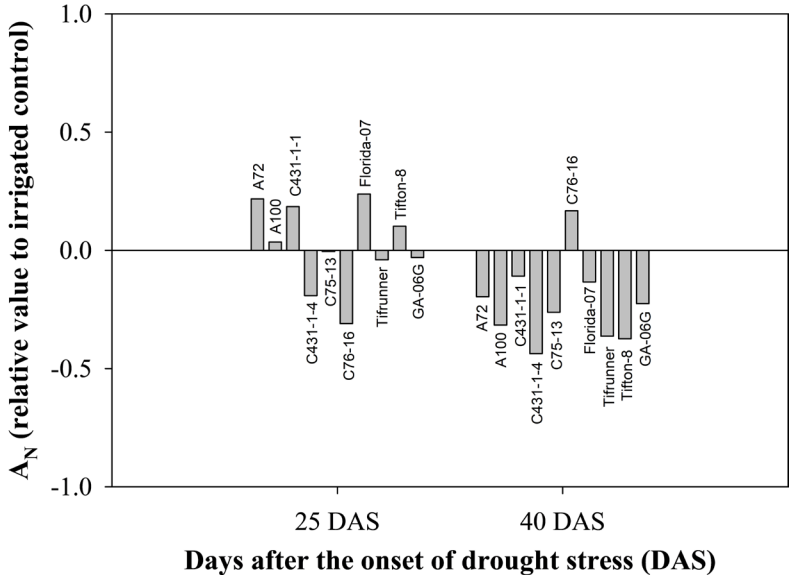

Figure 3. Relative values of net photosynthesis (A_N) between irrigated control and drought stress for ten peanut genotypes measured at 25 and 40 days after the onset of drought stress (DAS; or 59 and 74 days after planting). Values are average of five replicates and were calculated using the equation: Relative value = (A_N drought / A_N control) - 1.

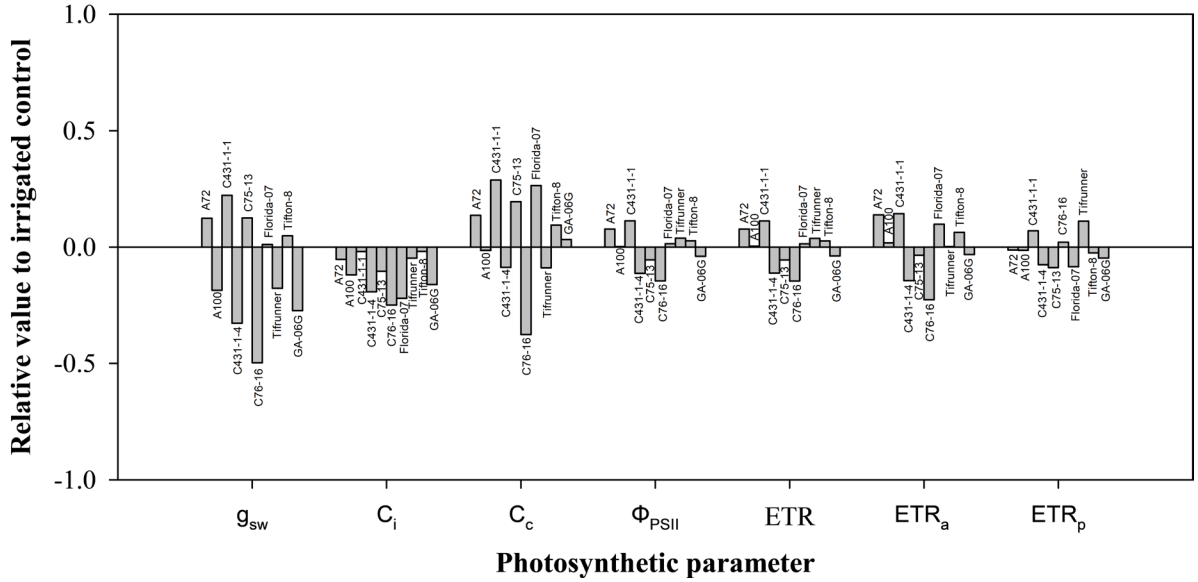

Figure 4. Relative values of multiple photosynthetic parameters between irrigated control and drought stress for ten peanut genotypes measured at 25 days after the onset of drought stress (DAS; or 59 days after planting (DAP)). Values were calculated using the equation: Relative value = ($PV_{drought}$ / $PV_{control}$) - 1, in which PV is the value for the parameter of interest. Photosynthetic parameters were: stomatal conductance (g_{sw}), intercellular CO_2 concentration (C_i), CO_2 concentration in the chloroplast stroma (C_c), quantum yield of Photosystem II (Φ_{PSII}), electron transport rate (ETR), electron transport flux to carbon assimilation (ETR_a), and electron transport flux to photorespiration (ETR_p). Each bar represents the average of five replicates for a peanut genotype within a given photosynthetic parameter.

In this study, although the non-stomatal parameters associated with the thylakoid reactions (Φ_{PSII}, ETR, ETR_a, and ETR_p) varied among the peanut genotypes, the variation had less magnitude compared to stomatal parameters under mild drought stress (Fig. 4). Some genotypes (e.g. Tifrunner) increased the relative value for ETR_p while maintaining a positive relative value for Φ_{PSII} and negative relative value for g_{sw}, suggesting a photoprotective response under drought conditions (Fig. 4). Studies on other species have reported a similar response of plants under water-deficit stress in which increasing photorespiration and dissipation of excess absorbed light through photochemical and non-photochemical processes were an adaptive mechanism used by the plants to avoid photoinhibition (Galmés et al., 2007; Meeks et al., 2019).

At 40 DAS, genotypes showed greater sensitivity to drought than 25 DAS (Fig. 5). Genotypes A72, A100, C75-13,

Florida-07, and Tifton-8 had increased flux of electrons allocated to photorespiration (ETR_P), resulting in decreased A_N (Figs. 3 and 5). C76-16 maintained slightly greater stomatal conductance as well as Φ_{PSII} and ETR (flux to both carbon assimilation and photorespiration) under drought stress (Fig. 5). Interestingly, under mild stress (25 DAS), this genotype had lower photosynthetic activity in plants grown under drought compared to those in irrigated conditions by downregulating multiple stomatal and non-stomatal components (Figs. 3 and 4). This reversible downregulation of the photosynthetic process, particularly the thylakoid reactions, may be associated with potential suppression of photoproduction of ROS (unstable molecules that damage cells). Downregulation of quantum yield of PSII is controlled by the xanthophyll cycle and proton gradient across thylakoid membranes, scavenging ROS and protecting cells from damage (Asada, 2006).

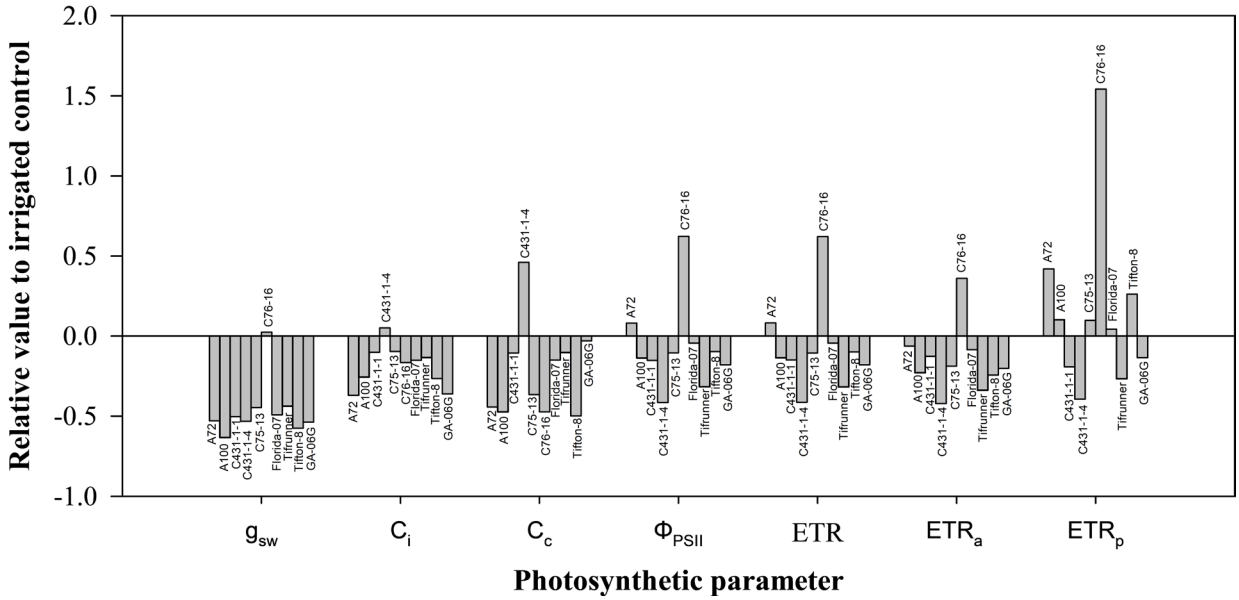

Figure 5. Relative values of multiple photosynthetic parameters between irrigated control and drought stress for ten peanut genotypes measured at 40 days after the onset of drought stress (DAS; or 7 days after planting (DAP)). Values were calculated using the equation: Relative value = ($PV_{drought}$ / $PV_{control}$) - 1, in which PV is the value for the parameter of interest. Photosynthetic parameters were: stomatal conductance (g_{sw}), intercellular CO_2 concentration (Ci), CO_2 concentration in the chloroplast stroma (C_c), quantum yield of Photosystem II (Φ_{PSII}), electron transport rate (ETR), electron transport flux to carbon assimilation (ETR_a), and electron transport flux to photorespiration (ETR_p). Each bar represents the average of five replicates for a peanut genotype within a given photosynthetic parameter.

Under severe drought (40 DAS), C76-16 responded differently, sustaining greater photosynthetic rates in plants under drought compared to full irrigation by upregulating stomatal activity and maintaining the thylakoid reactions (Φ_{PSII} and ETR), while increasing ETR_P to ensure photoprotection due to a decrease in C_c (Fig. 5). CO_2 concentration in the chloroplast stroma (C_c) is estimated to be approximately half of that in the ambient air, indicating great resistance to diffusion of CO_2 from ambient air surrounding the plant to the chloroplast stroma (Evans and Loreto, 2000). Therefore, it is not uncommon for the plants to have low C_c at "normal" stomatal conductance. The results suggest that photosynthetic apparatus in C76-16 had greater functionality in drought-stressed plants, with improved quantum yield of PSII and electron transport flux (Fig. 5). Electron transport flux was directed to both CO_2 assimilation (ETR_a) to generate NADPH for further use in the light-independent reactions of photosynthesis and photorespiration (ETR_p) to serve as a photoprotective mechanism, as previously described. This suggests that C76-16 has plasticity to regulate photosynthetic component processes required for thermal energy dissipation, photoprotection, and photosynthesis maintenance.

After assessing genotype response to progressive drought, Pearson's correlation analysis was used to identify the photosynthetic components that were more closely associated with A_N, and could potentially be used as surrogates for estimating photosynthetic performance of peanut genotypes under drought conditions. In addition, this knowledge can contribute in breeding efforts for targeted selection. Defining specific traits associated with improved photosynthesis under abiotic stress gives the possibility of transferring the trait by targeting the genetic code or gene region in control of that given trait. Stomatal conductance, Φ_{PSII}, ETR, and ETR_a were positively correlated with A_N at all times, regardless of stress timing or water regime (Table 2). When all data were combined and an overall correlation model was run, all photosynthetic parameters were significantly correlated with A_N, except for ETR_p. However, strong, positive correlations were observed only for g_{sw} and ETR_a, with correlation coefficients of 0.82 and 0.92, respectively. Therefore, linear regressions with A_N were obtained only for these two parameters.

Table 2. Pearson's correlation table between net photosynthesis and several photosynthetic components measured in irrigated and drought-stressed peanut plants at 25 and 40 days after the onset of drought stress (DAS; or 59 and 74 days after planting; DAP). Photosynthetic parameters were: stomatal conductance (g_{sw}), intercellular CO_2 concentration (C_i), CO_2 concentration in the chloroplast stroma (C_c), quantum yield of Photosystem II (Φ_{PSII}), electron transport rate (ETR), electron transport flux to carbon assimilation (ETR_a), and electron transport flux to photorespiration (ETR_p). Results from all then genotypes were pooled for this analysis. Values are correlation coefficients. * and ** indicate significance at 0.05 and 0.01 probability.

Photosynthetic parameter	25 DAS (or 59 DAP)		40 DAS (or 74 DAP)		Overall
	Irrigated	Drought	Irrigated	Drought	
g_{sw}	0.87**	0.90**	0.83**	0.57**	0.82**
C_i	0.30	0.73**	0.35	0.32	0.44**
C_c	0.90**	0.76**	0.02	0.18	0.47**
Φ_{PSII}	0.54**	0.76**	0.79**	0.63**	0.56**
ETR	0.54**	0.77**	0.79**	0.64**	0.56**
ETR_a	0.95**	0.94**	0.94**	0.92**	0.92**
ETR_p	-0.45*	0.23	0.45*	0.10	-0.16

The relationship between A_N and g_{sw} had a coefficient of determination of 0.66, indicating that 66% of the variation in A_N was accounted for by the g_{sw} (Fig. 6). Data points were more scattered under low A_N and g_{sw} regardless of water regime, lying closer to the fitting line as stomata were more open (Fig. 6). This model showed moderate accuracy using g_{sw} to predict A_N. Infrared gas analyzer systems designed for photosynthetic survey measurements, including A_N, are generally costly, require skilled labor for operation, and readings take between 2 to 3 minutes per sample. However, several user-friendly, light-weight porometer devices that measure g_{sw} are available at a lower cost, allowing for a broader range of users and requiring minimal training. In addition, sample readings take approximately 20-30 seconds, allowing for assessment of more samples within a given timeframe compared with more complex infrared gas analyzers. Stomatal conductance has been previously reported as an indicator of A_N rates and photosynthetic efficiency in peanut (Pilon et al., 2018) as well as in other species (Meeks et al., 2019; Medrano et al., 2002). Although the R^2 of the relationship between A_N and g_{sw} was not very strong (Fig. 6), g_{sw} could serve as a reference parameter in drought stress studies. The combination of g_{sw} with other photosynthetic parameters can strengthen the estimation of A_N and drought impact in the photosynthetic efficiency of peanut plants.

ETR_a was also plotted against A_N (Fig. 7). A strong, positive relationship was observed, regardless of genotype, water regime, or stress timing, with an $R^2 = 0.86$ (Fig. 7). For optimal A_N (30 µmol $m^{-2}s^{-1}$ or greater), ETR_a was predicted to be at least 160 µmol $m^{-2}s^{-1}$. Electron transport flux to CO_2 assimilation is crucial for NADPH formation and further use in the reduction stage of Calvin cycle for glucose production (Eberhard et al., 2008). The ratio of electron transport flux directed to CO_2 assimilation and photorespiration (ETR_a/ETR_p) varied from 0.85 to 3.5 (data not shown), indicating that more electrons were being directed to NADPH formation and consequently glucose production. Although genotypes varied in allocation of ETR to CO_2 assimilation *versus* photorespiration, particularly under drought stress (Figs. 4 and 5), ETR_a was strongly, directly associated with A_N, contributing to estimating alterations in A_N due to drought intensity. Some genotypes more readily utilized photorespiration as a protective response to drought, and even though different mechanisms exist for coping with drought among the genotypes, ETR_a was highly predictive of A_N across all genotypes and stress conditions.

Low water availability at reproductive development can limit A_N, decreasing growth and ultimately yield (Pilon et al., 2018; Zhang et al., 2022). Improving drought tolerance in peanut is a continuing effort in breeding programs. Due to the complexity of drought tolerance driven by multiple genetic controls, identifying mechanisms to which peanut plants cope with drought is crucial for the progress towards the development of cultivars with improved drought tolerance. This study contributes to enhancing knowledge on sensitivity of photosynthetic component processes in peanut genotypes to progressive drought stress.

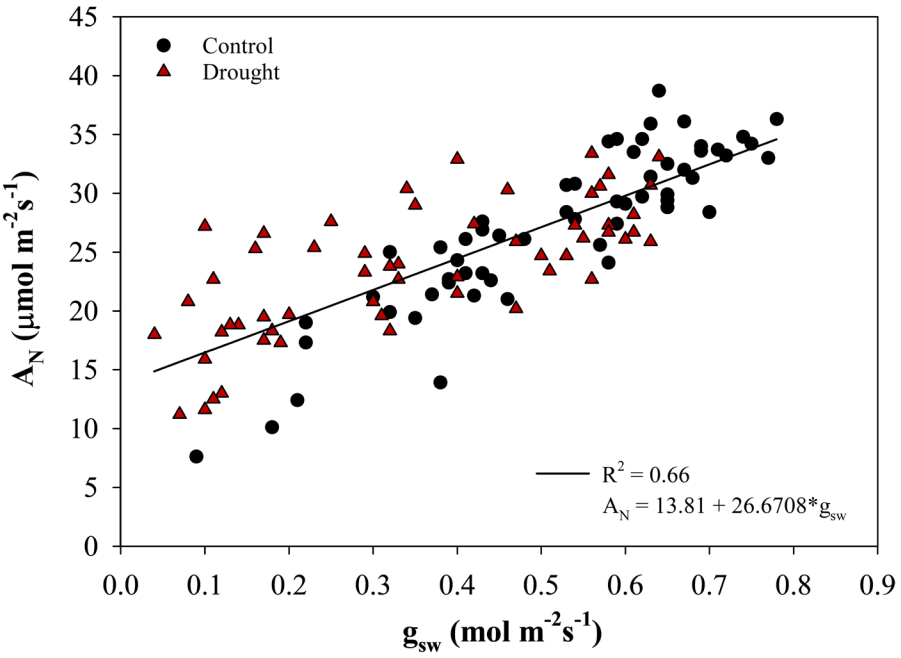

Figure 6. Linear response of net photosynthesis (A_N) to stomatal conductance (g_{sw}) in peanut. Data points represent measurements for the different genotypes grown under irrigated control (black circles) and drought stress (dark red triangles). Significant linear relationship between A_N and g_{sw} was observed ($P < 0.05$). Coefficient of determination and equation are presented in the graph.

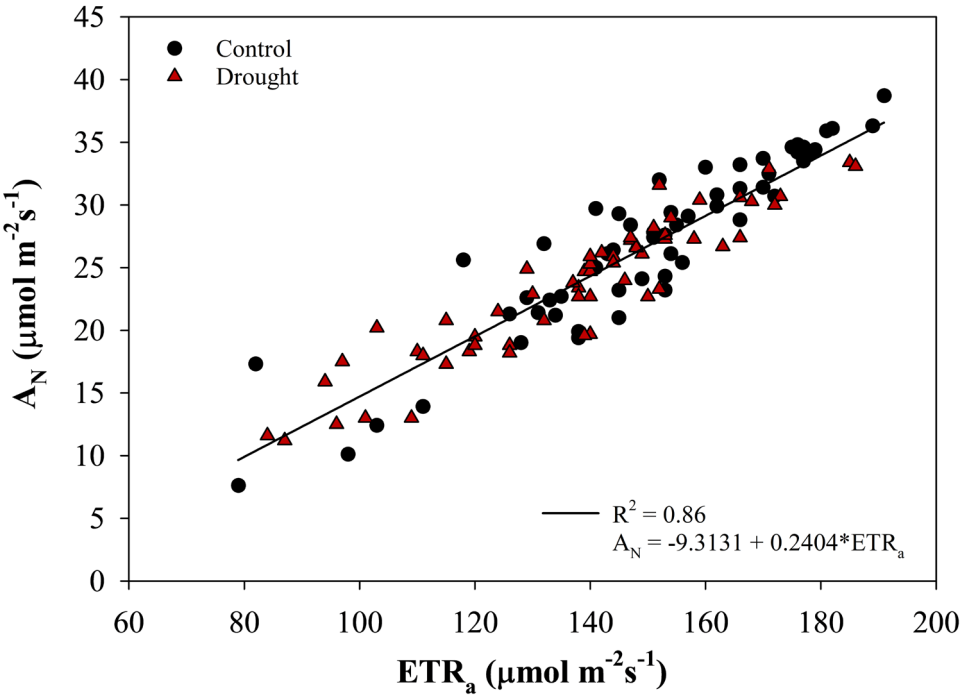

Figure 7. Linear response of net photosynthesis (A_N) to electron transport flux to CO_2 assimilation (ETR_a) in peanut. Data points represent measurements for the different genotypes grown under irrigated control (black circles) and drought stress (dark red triangles). Significant linear relationship between A_N and ETR_a was observed ($P < 0.05$). Coefficient of determination and equation are presented in the graph.

SUMMARY AND CONCLUSIONS

This study aimed to verify potential drought tolerance associated with the photosynthetic process within a diverse collection of ten peanut genotypes. C76-16 was identified as the most tolerant genotype due to improved plasticity and downregulation of photosynthesis under mild drought stress, whereas under more severe drought, multiple photosynthetic component processes were upregulated to sustain photosynthesis. This genotype was capable of maintaining increased stomatal conductance and controlling functionality of the thylakoid reactions while allocating electron flux to the photorespiratory pathway in response to low CO_2 concentration in the chloroplast stroma to prevent cell photodamage. With this study, another goal was to identify the primary limitation to carbon assimilation in the peanut genotypes under progressive drought during reproductive development. Stomatal conductance was the photosynthetic component process with greater variability within genotypes between the water regimes at mild drought, whereas non-stomatal factors (components within the thylakoid reactions) were nearly unaffected (or slightly impacted) by mild drought. Therefore, stomatal conductance was considered the primary factor limiting photosynthesis in peanut. Finally, the objectives of defining the photosynthetic parameter(s) with greatest contribution to photosynthetic drought tolerance as well as determining if the given parameter(s) can be used as reference indicator of photosynthesis response to drought intensity were also addressed. Stomatal conductance and electron flux to CO_2 assimilation contributed the most to drought tolerance in peanut genotypes. Moreover, these two photosynthetic component processes can be jointly used as reference indicators of photosynthetic status of peanut under different drought intensities.

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