Suitability of Canopy Temperature Depression, Specific Leaf Area, and SPAD Chlorophyll Reading for Genotypic Comparison of Peanut Grown in a Sub-humid Environment

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ABSTRACT

Peanut (*Arachis hypogaea* L.) is mostly grown under rainfed production with water deficit being the major limiting factor. Several physiological characteristics have been proposed as surrogates for yield and genotypic selection under water deficit in arid climates, but their suitability for selection under sub-humid rainfed production where water deficit can also occur is not clear. Canopy temperature depression (CTD), specific leaf area (SLA), and SPAD chlorophyll reading of eight virginia-type peanut genotypes were evaluated at three growth stages in field trials involving rainfed and irrigated plants in sub-humid environments in northeastern Virginia-Carolina (VC) region in 2011 and 2012. Significant ($p \leq 0.05$) variation in pod yield and all physiological characteristics was observed in response to water regime in both years. Rainfed plants had warmer (CTD 2.2 *vs.* 3.1 °C) and greener canopies in 2011 but cooler (CTD 3.9 *vs.* 2.2 °C) and less green canopies in 2012 than the irrigated plants. Compared to irrigated plants, rainfed plants had slightly increased SLA in 2011 (135 *vs.* 131 $cm^2\ g^{-1}$), but decreased SLA in 2012 (133 *vs.* 144 $cm^2\ g^{-1}$). Differences ($p \leq 0.05$) among genotypes were observed for pod yield, SLA, and SPAD chlorophyll reading, but not for CTD. Among the physiological characteristics, only SPAD chlorophyll readings were significantly correlated to pod yield in all water regimes and growth stages in 2012, but not in 2011. Based on these results, CTD, SLA, and SPAD chlorophyll reading appear to be unsuited for genotypic selection for yield and water-deficit tolerance for peanut grown in sub-humid environment of the Virginia-Carolina region in part due to unpredictable rainfall amount and distribution. For reproducible field evaluations, additional methods will have to be used such as use of rain exclusion shelters.

Key Words: Drought, rainfed, water deficit, Virginia market type, Virginia-Carolina region.

Peanut (*Arachis hypogaea* L.) is the fourth major oilseed crop grown worldwide after soybean [*Glycine max* (L.) Merr.], rapeseed (*Brassica napus* L.) and cottonseed (*Gossypium hirsutum* L.) (USDA FAS 2012) and represents a major source of protein and oil in the human diet in developing countries. In 2011, peanut was grown on 21.7 million ha with a total production of 38.6 MMT worldwide (FAOSTAT 2013). With 444,190 ha and 1.65 MMT of production in 2011 (FAOSTAT 2013), the United States was the fourth largest producer of peanut after China, India, and Nigeria. The major peanut producing states in the US are Georgia, Alabama, Texas, Florida, North Carolina and South Carolina. It is also important to note that most of the peanut production in the world is in arid or semi-arid climates, and peanut therefore is rainfed rather than irrigated.

Virginia-type peanut is an important crop for the Virginia-Carolina (VC) region comprising Virginia, North Carolina, and South Carolina. It is a ''cash'' crop grown annually on over 50,000 to 76,000 ha in all three states bringing important revenues to growers. Historically, with the incident average precipitation of 784 mm during the growing season in this region, the peanut production of over 250,000 tons per year can be easily achieved without supplemental irrigation. This is also why over 85% of the current peanut farming in the VC region is rainfed rather than irrigated. However, depending on soil type and geography, parts of the VC region have experienced inadequate rainfall and temperature extremes during the growing season in some years. In addition, fine sandy loam soils that are predominantly used for peanut production have poor water holding capacity, exacerbating water deficit stress. For example, in 2009, in general a rainy year with mean monthly rainfall of 103 mm,

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reduced precipitation during June (87 mm) reduced plant growth and resulted in decreased yields (Balota *et al.*, 2012a). Similarly, due to high temperatures and significantly reduced precipitation in 2010, yield and kernel quality were drastically reduced in the VC region (Balota *et al.*, 2012a). Adoption of irrigation is unlikely due to already high peanut production costs in Virginia and the Carolinas and limited water supply. Therefore, increasing yield under drought and heat in the VC region can only be achieved by the development of drought and heat tolerant cultivars.

Peanut crop can be susceptible to drought and high temperature stress (Bell *et al.*, 1994). Temperature extremes and drought stress individually or combined negatively influence the physiological fitness of plants, pod and seed quality, and yield. As such, there is incentive for development of cultivars with superior drought tolerance. This goal can be achieved by understanding how peanut plants successfully cope with drought stress, or alternatively; how some genotypes more successfully cope with drought stress than others.

In recent years, several physiology-based tools were developed to measure the relative levels of stress experienced by plants under drought stress. These tools are valuable for screening germplasm for drought stress tolerance. Some of the most commonly used screening tools include measuring chlorophyll content, specific leaf area (SLA), and canopy temperature depression (Reynolds *et al.*, 2009; Upadhyaya, 2005).

Water use efficiency (WUE) is the total biomass produced per unit of water transpired, and greater water use efficiency in crops is often associated with good adaptation to water stress. Specific leaf area and leaf chlorophyll content measured by soil plant analysis development (SPAD) chlorophyll meter were reported as good surrogates to select peanut genotypes with improved water use efficiency. Significant correlations were also reported among SPAD chlorophyll reading, specific leaf area and specific leaf nitrogen in peanut (Nageswara Rao et al., 2001). Sheshshayee et al. (2006) reported a strong and positive relationship between SPAD chlorophyll reading and WUE. Recent studies have shown that genetic variation for SPAD chlorophyll reading exists in peanut mini-core entries (Upadhyaya, 2005). Because of the correlation between chlorophyll and N content, SPAD chlorophyll readings alone can be used to estimate nitrogen content in several economically important crops (Chapman and Barreto, 1997, Rorie *et al.*, 2011). As such, SPAD chlorophyll reading was recommended as an appropriate selection criterion for screening for improved drought tolerance in peanut because of its high heritability and simplicity (Songsri *et al.*, 2009; Upadhyaya, 2005).

Plants employ various physiological mechanisms to avoid stress. Drought-tolerant cultivars tend to maintain cooler canopies during stress periods (Ayeneh *et al.*, 2002). Canopy temperature measured by infrared thermometers above the crop canopies is gaining wide acceptance as a potential selection tool for improved drought tolerance and crop yields (Balota *et al.*, 2008; Blum *et al.*, 1988; O'Toole *et al.*, 1984). The canopy temperature depression (CTD) method was described as a rapid, non-destructive, and non-disruptive method of germplasm screening for drought stress and yield (Balota *et al.*, 2012; O'Toole *et al.*, 1984).

Traits like CTD, SPAD chlorophyll reading, and SLA were successfully used as surrogates to identify drought and heat tolerant cultivars in other peanut growing areas of the world. However, very limited research in relation to drought of the virginia-type peanut is available in the sub-humid VC-region. This study aimed to evaluate the suitability of these physiological traits as surrogates for selection of virginia-type peanut cultivars and breeding lines under rainfed and irrigated field trials in the sub-humid VC-region of the United States.

Materials and Methods

Plant Materials and Growth Conditions. Eight peanut lines including commercial cultivars Bailey (Isleib *et al.*, 2011), CHAMPS (Mozingo *et al.*, 2006), and Phillips (Isleib *et al.*, 2006) and advanced breeding lines N04074FCT, N05006, N05008, N05024J, and SPT 06-07 were selected from the Peanut Variety and Quality Evaluation (PVQE) program based on the variability for CTD, SLA, and SPAD chlorophyll content under drought conditions (Balota *et al.*, 2012a, 2012b).

Field experiments were conducted at the Tidewater Agric. Res. and Extension Ctr., Suffolk, VA (36°68′N, 76°77′W, 25 m elevation) in 2011 and 2012 under rainfed and irrigated conditions. The experiment was a split-split plot design with whole plots arranged in randomized complete blocks (RCB). Water regime was the whole plot factor, genotype was the sub-plot factor, and growth stage a repeating measure factor. Measurements were taken on a fully expanded third youngest leaf arising from the main stem at three peanut growth stages, beginning flower (R1), beginning pod growth (R3), and beginning seed growth (R5) (Boote, 1982). Canopy temperature depression

was not measured at stage R1 in 2011 and R3 in 2012. Individual plots of 22 m^2 (12.2 m each row and 0.91 m between rows) and with approximately 9 seeds m^{-1} of row were treated as the experimental unit. Cultural practices were performed according to the Virginia Peanut Production Guide (http://www.ext.vt.edu). Air temperature, RH, rainfall, and photosynthetically active radiation (PAR) were continuously monitored next to the plots with a weather station (WatchDog Temperature/RH Station, Model 2450, Spectrum Technologies Inc., Plainfield, IL), in both years. In 2011, 25.4 mm of irrigation was supplied to the irrigated plots on 24 June, 31.8 mm on 1 July, and 22.9 mm on 19 July using a lateral pull boom cart sprinkler irrigation system (E1025 Reel Rain, Amadas Ind., Suffolk, VA). In 2012, plots in the irrigated water regime received 16.5 mm irrigation on 28 June and 15.8 mm on 18 July. Pod yield data were collected at the physiological maturity in both years.

Canopy Temperature Depression. The CTD of each plot was measured with a hand-held infra-red thermometer (IRT) (Agri-Therm Model 6110L, Everest Interscience Inc., Tucson, AZ) twice at the center of each row in two rows, a total of four readings per plot, at approximately 50 cm above the canopy. The CTD was measured as the difference between canopy temperature (T_c) and air temperature (T_a) and converted to positive values when canopies were cooler than the air by multiplying the Agri-Therm readings −1.0, i.e. with the formula:

$$\mathrm{CTD} = -1\ (T_\mathrm{c} - T_\mathrm{a}) \quad (1)$$

Specific Leaf Area and SPAD Chlorophyll Reading. Four fully expanded third youngest leaves from the main stem per plot were collected in the field and transported to laboratory in moistened paper towels for measurements of leaf area using a LI-3000 leaf area meter (LICOR Inc., Lincoln, NE) and for relative chlorophyll content by the SPAD (Soil Plant Analysis Development) chlorophyll reading (SPAD-502, Konica Minolta Optics Inc., Japan). SLA was calculated after weighing the dried leaves in an oven at 65°C for 24 hours using the formula:

$$\mathrm{SLA} = \text{Leaf area } \left(\mathrm{cm}^2\right) / \text{leaf dry weight (g)} \quad (2)$$

Statistical Analysis. Individual plots were treated as the experimental unit and measurements were taken at three peanut growth stages at beginning flower (R1), beginning pod (R3), and beginning seed (R5). Data from CTD, SLA, and SPAD chlorophyll reading were analyzed as a repeated measures design in a split-split plot arrangement, with growth stage as a repeated measure factor. The univariate model for the repeated measures data was:

$$y_{jkl} = \mu + w_j + g_k + s_l + (wg)_{jk} + (ws)_{jl} + (gs)_{kl} + (wgs)_{jkl} + e_{jkl} \quad (3)$$

where μ is the overall mean effect, w_j the main effect of the j^{th} water regime (j = 1, 2), g_k the main effect of the k^{th} genotype (k = 1 to 8), s_l the growth stage effect (l = 1 to 3), $(wg)_{jk}$ the interaction of the j^{th} water regime and k^{th} genotype, $(ws)_{jl}$ the interaction of the j^{th} water regime and l^{th} growth stage, $(gs)_{kl}$ the interaction of the k^{th} genotype and l^{th} growth stage, $(wgs)_{jkl}$ the interaction of the j^{th} water regime, k^{th} genotype, and l^{th} growth stage, and e_{jkl} the random error associated with the $ijkl^{\mathrm{th}}$ experimental unit. Genotypes and water regimes were treated as fixed effects while the effects of repeated measures, *i.e.*, growth stage, was considered to be random. The sphericity requirements of a univariate repeated measures analysis were tested and then adjusted accordingly using Greenhouse-Geisser (G-G) and Huynh-Feldt (H-F) corrections (Collaku and Harrison, 2002). The data were analyzed using MANOVA option for repeated measures on JMP software (SAS Institute Inc., Ver. 10.0, Cary, NC, 2012).

Correlations between physiological and agronomic traits were calculated. Pod yield was measured at the end of the season and the data analyzed as a split plot design. All the treatments were considered to be fixed in the ANOVA model:

$$Y_{jk} = \mu + w_j + g_k + (wg)_{jk} + e_{jk} \quad (4)$$

where μ is the overall mean effect, w_j the main effect of the j^{th} water regime (j = 1, 2), g_k the main effect of the k^{th} genotype (k = 1 to 8), $(wg)_{jk}$ the interaction of the j^{th} water regime and k^{th} genotype, and e_{jk} the random error associated with the experimental unit.

For all traits, mean differences for water regimes and genotypes within a year and growth stage were calculated by Student's t-test ($p \leq 0.05$) and Tukey's HSD test ($p \leq 0.05$).

Results

Years 2011 and 2012 in general and sampling times (June through August each year) in particular were different from each other in terms of temperature, rainfall, relative humidity (RH), and photosynthetically active radiation (Table 1). Overall, the

Table 1. Maximum temperature, total rainfall, relative humidity, and photosynthetically active radiation during June–August in Suffolk, VA in 2011 and 2012. For comparisons, long-term (1933–2012) maximum temperature and rainfall are also presented.

	Maximum temperature (C)			Rainfall (mm)			RH (%)		PAR (μmoles m^{-2} s^{-1})	
Week	2011	2012	1933–2012	2011	2012	1933–2012	2011	2012	2011	2012
wk-2 June	33.9	29.7	29.7	41.7	10.9	25.3	71	73	1846	1204
wk-3 June	31.3	29.4	29.8	9.9	10.7	24.4	71	76	1609	1262
wk-4 June	33.8	32.8	31.1	26.9	84.3	31.7	75	73	1767	1203
wk-1 July	34.6	36.6	31.1	43.7	5.6	24.1	74	72	1895	1290
wk-2 July	34.0	31.0	31.2	13.7	44.7	36.2	76	84	1914	904
wk-3 July	35.2	34.1	31.7	0.0	47.8	34.5	68	80	1754	1224
wk-4 July	36.9	33.6	31.6	116.3	22.4	50.5	75	84	1700	1190
wk-1 August	33.9	32.0	31.3	3.6	52.6	35.5	83	85	1584	1020
wk-2 August	33.5	31.4	30.8	15.2	33.5	33.8	75	86	1574	929
Average	34.1	32.3	30.9	271.0	312.4	296.0	74	79	1738	1136

[a]Abbreviations. Photosynthetically active radiation, PAR. Relative humidity, RH.

2011 growing season was hot, dry with high maximum temperatures (34.1 C), and relatively low precipitation (271 mm) from June to August. Visible symptoms of water deficit stress, *i.e.*, leaf wilting, were observed at stages R1 and R3 in 2011. Later in the growing season, a record of 346 mm rainfall occurred on 27 August, and with repeated rainfall occurring in subsequent days. The 2012 growing season was relatively warm and humid with mean maximum temperatures around 32 C, total rainfall 312 mm, and mean RH of 79% during the June through August interval. During this time, in June specifically, rainfed plants produced less biomass than irrigated plants based on visual comparison, but they were not wilted. From July through October, plots received weekly precipitation, with total rainfall from May to October of 965 mm, approximately 60% more than the multi-annual historical precipitation. Due to persistent cloud cover throughout the entire growing season, photosynthetically active radiation was 30% less in 2012 than in 2011.

Pod Yield. ANOVA for pod yield showed significant main effects of water regime and genotype in both years, and a significant water regime-by-genotype interaction effect in 2012 (Table 2). In 2011, rainfed plots yielded less than irrigated plots, which received an additional 80-mm water through irrigation during June and July (5783 *vs.* 6128 kg ha^{-1}, $p \leq 0.05$). In 2012, average rainfed plots were higher in yield than irrigated plots (4359 *vs.* 4185 kg ha^{-1}, $p \leq 0.05$) probably due to reduced radiation and excessive moisture on the irrigated plots during the latter part of the 2012 season. N04074FCT had the lowest average pod yield and N05008, N05024J, and Phillips the highest among the genotypes tested under rainfed conditions during both years (Table 3). N05006 had ranked third greatest for yield in 2012, but performed poorly in 2011, the drier year. Low yields under rainfed conditions were recorded for CHAMPS, Bailey, and SPT 06-07 in both years. N05008 had the highest rainfed yield in 2011, but its yield reduction was 8% as compared to its irrigated yield (6360 *vs.* 7147 kg ha^{-1}). Bailey, CHAMPS, N04074FCT and SPT 06-07 had the greatest reduction of average yield (6.7 to 7.9%), and Phillips and N05006 the lowest (3.9 and 5.5%, respectively) due to water deficit in 2011. In 2012, irrigated SPT 06-07 had a reduction of 11.2% in pod yield compared with its rainfed yield. For all other genotypes yield was higher in rainfed than in irrigated plots in 2012 (Table 3).

Canopy Temperature Depression. Univariate repeated measures ANOVA of CTD showed significant water regime and growth stage-by-water regime interaction effects in 2011 and 2012 (Table 4). Growth stage effects were highly significant in 2011 ($p \leq 0.001$) and were absent in 2012. In 2011, irrigated and rainfed plants were similarly cooler relative to air temperature (2.4°C) at stage R3, but irrigated plants became significantly cooler than rainfed plants later in the season, at stage R5

Table 2. Analysis of variance for pod yield of eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) in 2011 and 2012.

		F-ratio	
		Pod yield	
Source of variation	df[a]	2011	2012
Water regime (W)	1	5.24*	4.46*
Genotype (G)	7	5.44***	13.14***
G × W	7	0.23	2.36*

[a]Abbreviations. Degrees of freedom, df; ***, **, * Significant at the 0.001, 0.01 and 0.05 probability levels, respectively.

Table 3. Pod yield and reduction of eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) in 2011 and 2012.

Genotype	2011			2012		
	Rainfed	Irrigated	Reduction	Rainfed	Irrigated	Reduction
	kg ha^{-1}		%	kg ha^{-1}		%
Bailey	6185 ab[a]	6713 a	−7.9	4283 a	4231 ab	1.2
CHAMPS	5563 ab	6007 ab	−7.4	4273 ab	3969 a–c	7.7
N04074FCT	5072 b	5436 ab	−6.7	3440 bc	3203 c	7.4
N05006	5734 ab	6069 ab	−5.5	4685 a	4365 a	7.3
N05008	6360 ab	6888 a	−8.1	4642 a	4391 a	5.7
N05024J	6029 ab	5930 ab	1.7	4696 a	4696 a	0.0
Phillips	6193 ab	6442 ab	−3.9	4765 a	4020 a–c	18.5
SPT 06-07	5125 b	5539 ab	−7.5	4091 ab	4607 a	−11.2
Mean	5783	6128	−5.6	4359	4185	4.2

[a]Means followed by different lower-case letters are significantly different for genotype by water regime within each year (P≤0.05 Tukey-HSD).

(p≤0.05) (Table 5). In 2012, rainfed plots were on average 1.7°C cooler than irrigated canopies during R1 and R5 growth stages. Within each growth stage and water regime, there were no significant differences for CTD among genotypes in both years.

Specific Leaf Area and SPAD Chlorophyll Reading. Genotype, water regime, and growth stage affected (p≤0.05) specific leaf area (SLA) and SPAD chlorophyll reading both years (Table 4). During both years, the growth stage by water regime interaction was significant (p≤0.05) for SLA and

Table 4. Univariate repeated measures analysis of canopy temperature depression (CTD), specific leaf area (SLA), and SPAD chlorophyll reading of eight peanut genotypes under two water regimes in 2011 and 2012.

Source of variation	2011		2012	
	df[a]	F-ratio[b]	df	F-ratio
CTD				
Genotype (G)	7	1.20	7	1.69
Water regime (W)	1	113.93***	1	148.20***
G × W	7	1.10	7	0.46
Growth stage (GS)	1	23.03***	1	0.16
GS × G	7	0.55	7	0.51
GS × W	1	76.79***	1	9.16**
GS × G × W	7	0.68	7	0.47
SLA				
Genotype (G)	7	3.17**	7	3.54**
Water regime (W)	1	6.99*	1	27.67***
G × W	7	0.32	7	0.96
Growth stage (GS)	2	106.56***	2	91.47***
GS × G	14	1.71	14	2.61**
GS × W	2	3.64*	2	9.39***
GS × G × W	14	0.73	14	1.29
SPAD Chlorophyll				
Genotype (G)	7	39.76***	7	11.35***
Water regime (W)	1	67.22***	1	11.52**
G × W	7	1.13	7	2.15
Growth stage (GS)	2	57.98***	2	494.99***
GS × G	14	4.46***	14	1.54
GS × W	2	51.08***	2	4.36*
GS × G × W	14	1.01	14	0.92

[a]Abbreviations. Degrees of freedom, df; ***, **, * Significant at the 0.001, 0.01 and 0.05 probability levels, respectively.
[b]The adjusted univariate Greenhouse-Geisser (G-G) and Huynh-Feldt (H-F) F-ratio.

Table 5. Canopy temperature depression (CTD) of eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) at two growth stages (R3, beginning pod; and R5, beginning seed) in 2011 and at three growth stages (R1, beginning flower and R5, beginning seed) in 2012 under field conditions.

	2011				2012			
	R3		R5		R1		R5	
Genotype	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated
	°C							
Bailey	2.32 ab[a]	2.22 ab	2.40 a–d	3.44 a–d	3.39 abc	2.11 abc	3.71 abc	2.23 f
CHAMPS	2.29 ab	2.01 b	1.73 d	4.08 a	3.78 abc	1.87 bc	3.63 a–e	2.46 def
N04074FCT	2.39 ab	2.59 ab	2.51 a–d	4.01 a	4.61 ab	2.05 bc	3.70 abc	2.74 a–f
N05006	2.09 ab	2.36 ab	1.75 d	3.94 ab	4.64 ab	2.37 abc	3.68 a–d	2.59 b–f
N05008	2.57 ab	2.33 ab	1.70 d	3.71 abc	4.16 abc	1.40 c	3.48 a–e	2.13 f
N05024J	2.23 ab	2.63 ab	1.74 d	3.98 a	4.88 a	2.09 abc	3.81 ab	2.57 c–f
Phillips	2.59 ab	2.70 ab	2.14 bcd	3.88 ab	4.20 abc	1.87 bc	3.89 a	2.59 b–f
SPT 06-07	2.78 a	2.57 ab	1.95 cd	3.80 ab	3.14 abc	1.60 c	3.57 a–e	2.43 ef

[a]Means followed by different lower-case letters are significantly different for genotype × water regime within each growth stage and year ($P \leq 0.05$ Tukey-HSD).

SPAD chlorophyll reading. In both years, the smallest SLA values were recorded at R3 (beginning pod) and the greenest leaves were at R5 (beginning seed) stages (Tables 6 and 7). Overall, leaves of rainfed plants had higher SLA (135 *vs.* 131 $cm^2\ g^{-1}$) in 2011 and smaller SLA (133 *vs.* 144 $cm^2\ g^{-1}$) in 2012 than the corresponding irrigated plants (Table 8). A similar pattern was observed for the SPAD chlorophyll readings. In 2011, average SPAD chlorophyll readings were 47.4 and 54.3 (relative units) for the rainfed and irrigated plants, respectively. In 2012, these numbers corresponded to 42.7 and 44.1 (relative units) for the rainfed and irrigated plants, respectively.

With a few exceptions, Bailey and CHAMPS had lower and Phillips higher SLA than other genotypes in both growing seasons (Table 6). For N04074FCT, SLA was relatively high at early stages and low at stage R5 in 2011 and 2012. N05006 and SPT 06-07 had lower SLA early than late in the season. Among all lines and based on the SPAD chlorophyll readings, SPT 06-07 was the greenest and N04074FCT the least green in both years and at all growth stages (Table 7).

Discussion

Pod Yield. Although soil moisture was adequate in 2011, *i.e.*, total rainfall from May through October was 783 mm excluding the rainfall received from the tropical storm Irene, rainfed plots yielded on average 6% less than irrigated plots with a range of reduction due to water-deficit from less than 5% for Phillips and N05006 to approximately 7% for N05008, Bailey, CHAMPS, SPT 06-07, and N04074FCT (Table 3). The 2012 growing season was wetter than that of 2011 and quite hot based on historical data (Table 1). In addition to the 965 mm rainfall from May to October for all plots, irrigated plots received additional 32.3 mm irrigation from end of June to mid-July in 2012. Reduced radiation and excessive moisture could have contributed to lower yields in irrigated than in rainfed plots in 2012 as well as a 28% decrease in yield in 2012 compared to 2011 (Table 3).

Average pod yields varied significantly for different genotypes under rainfed conditions in both years. N05008, Phillips, and Bailey were the top yielding lines under rainfed conditions in 2011, while Phillips, N05024J, N05006, and N05008 were in 2012. In 2012, all genotypes showed increases in pod yield under rainfed conditions excepting only SPT 06-07, for which pod yield was significantly reduced (11.2%) under rainfed conditions. The lowest yielding lines in rainfed plots in both years were N04074FCT, CHAMPS, and SPT 06-07. Greenhouse studies of recovery from drought demonstrated that SPT 06-07 and N05006 recovered well from severe drought, while N04074FCT and Bailey showed a poor recovery from drought stress (Rosas-Anderson, 2012). Our field results are in an agreement with the greenhouse findings for N04074FCT and N05006, but not for Bailey and SPT 06-07. The discrepancies for Bailey and SPT 06-07 could be the result of their different yield potentials. Bailey has high yield potential that could have allowed this line to yield well under rainfed water regime even if the decrease from its irrigated yield was substantial, 7.9%. On the other hand, SPT 06-07 has inherently low yield potential, for which a 7.5% rainfed yield reduction could have made it the second lowest for yield after N04074FCT. Overall, Phillips, N05008, and

Table 6. Specific leaf area (SLA) of eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) at three growth stages (R1, beginning flower; R3, beginning pod; and R5, beginning seed) in year 2011 and 2012 under field conditions.

	2011						2012					
	R1		R3		R5		R1		R3		R5	
Genotype	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated
	$cm^2\ g^{-1}$											
Bailey	146.0 a[a]	159.0 a	115.9 bc	113.2 c	128.9 ab	118.1 b	161.4 ab	157.2 ab	109.6 de	106.4 e	126.4 b	129.7 b
CHAMPS	146.0 a	143.0 a	112.0 c	113.7 c	129.9 ab	122.8 ab	132.0 ab	163.2 ab	111.2 de	111.9 c–e	132.4 ab	135.0 ab
N04074FCT	149.0 a	161.7 a	133.3 a	125.5 a–c	134.0 ab	123.5 ab	153.7 ab	180.4 a	118.3 b–e	119.4 b–e	136.3 ab	137.8 ab
N05006	145.4 a	139.4 a	125.0 a–c	115.6 bc	133.9 ab	128.1 ab	116.1 b	162.4 ab	128.3 ab	124.0 a–d	139.2 ab	146.1 ab
N05008	142.7 a	147.5 a	123.1 a–c	114.8 c	135.9 ab	129.4 ab	143.9 ab	156.5 ab	123.3 b–d	123.2 b–d	125.9 b	144.9 ab
N05024J	149.1 a	154.3 a	132.6 ab	122.8 a–c	135.3 ab	129.0 ab	139.0 ab	166.9 a	131.6 ab	129.7 ab	140.2 ab	156.5 ab
Phillips	155.7 a	158.8 a	121.3 a–c	116.6 a–c	140.5 a	126.1 ab	143.7 ab	158.1 ab	123.0 b–d	127.4 a–c	137.4 ab	156.9 ab
SPT 06-07	148.1 a	135.5 a	115.2 c	115.8 bc	142.1 a	135.6 ab	147.6 ab	169.4 a	121.3 b–e	139.3 a	145.5 ab	163.3 a

[a]Means followed by different lower-case letters are significantly different for genotype × water regime within each growth stage and year (P≤0.05 Tukey-HSD).

Table 7. The SPAD chlorophyll reading of eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) at three growth stages (R1, beginning flower; and R5, beginning seed) in years 2011 and 2012 under field conditions.

	2011						2012					
	R1		R3		R5		R1		R3		R5	
Genotype	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated
	relative units											
Bailey	44.0 bc[a]	42.6 bc	49.5 a–d	44.1 fg	47.2 c–f	48.9 a–d	34.5 bc	38.5 a–c	42.9 b–d	43.0 b–d	46.5 b	49.5 ab
CHAMPS	45.0 bc	43.8 bc	50.1 a–d	44.4 e–g	46.3 c–g	47.3 b–f	36.6 bc	39.4 a–c	43.1 b–d	43.3 b–d	46.6 b	48.9 ab
N04074FCT	42.7 bc	41.3 c	47.9 b–f	42.5 g	44.6 e–h	42.6 h	33.9 bc	32.8 c	40.7 d	41.8 cd	46.3 b	47.8 b
N05006	46.2 b	44.1 bc	50.3 a–c	46.0 c–g	49.8 a–c	48.0 a–e	40.9 ab	36.9 bc	46.2 ab	45.2 a–c	51.1 ab	51.4 ab
N05008	45.5 b	43.4 bc	50.7 ab	45.7 c–g	47.9 b–e	49.5 a–c	36.6 bc	38.7 a–c	44.8 a–d	44.8 a–d	47.3 b	49.9 ab
N05024J	43.7 bc	44.5 bc	49.4 a–d	45.5 d–g	43.6 gh	44.0 f–h	37.0 bc	36.5 bc	43.1 b–d	42.0 b–d	46.1 b	48.7 b
Phillips	44.5 bc	43.7 bc	48.2 b–f	41.8 g	45.5 d–h	44.2 f–h	36.4 bc	39.3 a–c	42.3 b–d	43.3 b–d	47.0 b	49.1 ab
SPT 06-07	50.8 a	50.2 a	53.1 a	49.0 a–e	50.8 ab	51.4 a	40.6 ab	44.8 a	45.2 a–c	48.1 a	48.6 b	54.1 a

[a]Means followed by different lower-case letters are significantly different for genotype × water regime within each growth stage and year (P≤0.05 Tukey-HSD).

Table 8. Mean specific leaf area (SLA) and SPAD chlorophyll reading of eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) at three growth stages (R1, beginning flower; R3, beginning pod; and R5, beginning seed) in year 2011 and 2012 under field conditions.

	2011						2012					
	R1		R3		R5		R1		R3		R5	
Trait	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated
SLA	$cm^2\ g^{-1}$											
	147.7 A[a]	149.9 A	122.3 A	117.3 B	135.1 A	126.6 B	142.2 B	164.3 A	120.8 A	122.7 A	135.4 B	146.3 A
SPAD	relative units											
	45.3 A	44.2 B	49.9 A	44.9 B	47.0 A	46.9 A	37.1 A	38.4 A	43.5 A	43.9 A	47.5 B	49.9 A

[a]Means followed by different capital letters between water regimes and within a growth stage are significantly different ($P \leq 0.05$ student's t-test).

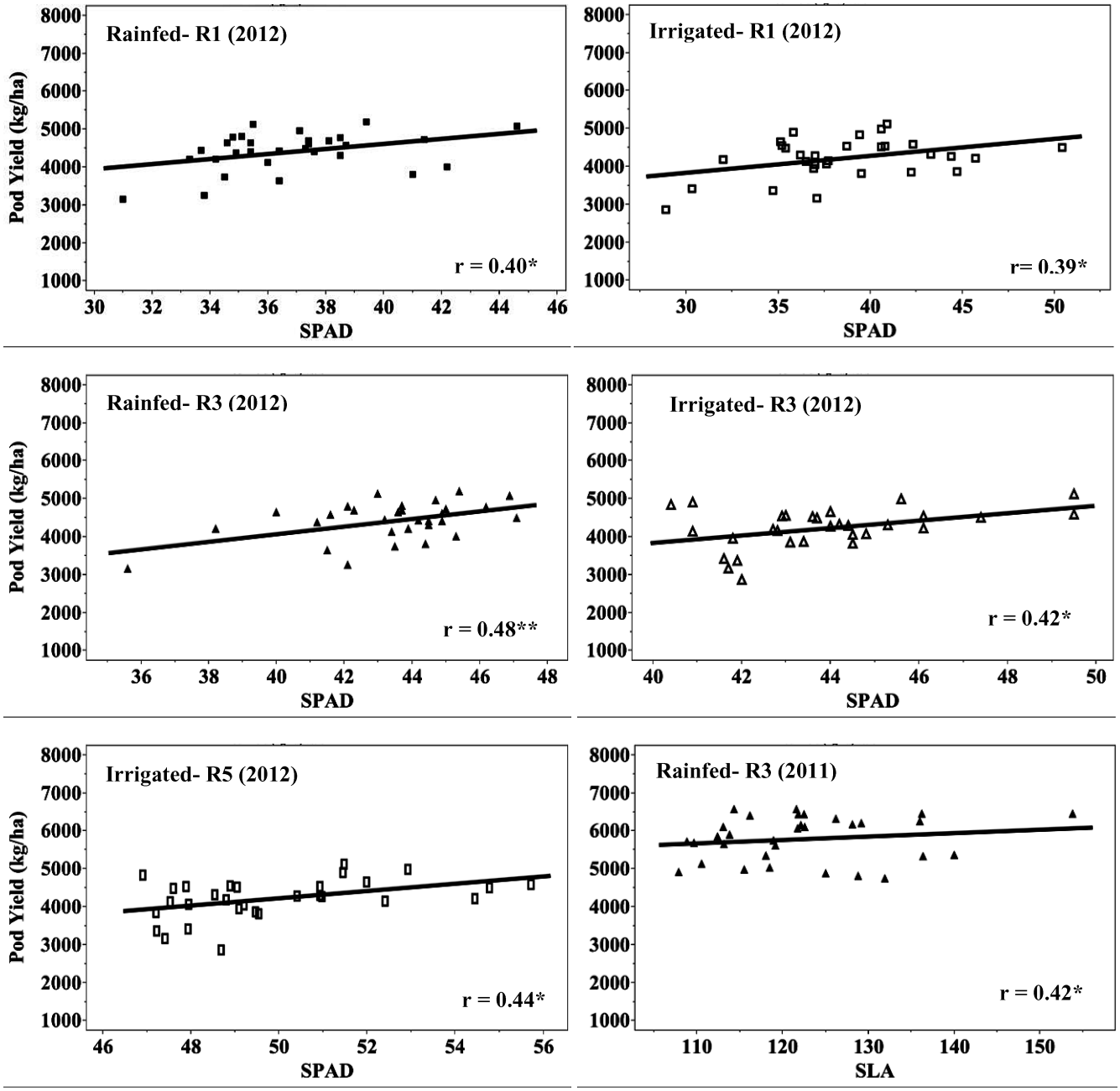

Fig. 1. Correlation of pod yield, SPAD chlorophyll content, and SLA for eight virginia-type peanut genotypes at three growth stages (R1, beginning flower; R3, beginning pod; and R5, beginning seed), two water regimes (rainfed and irrigated) during field conditions in 2012.

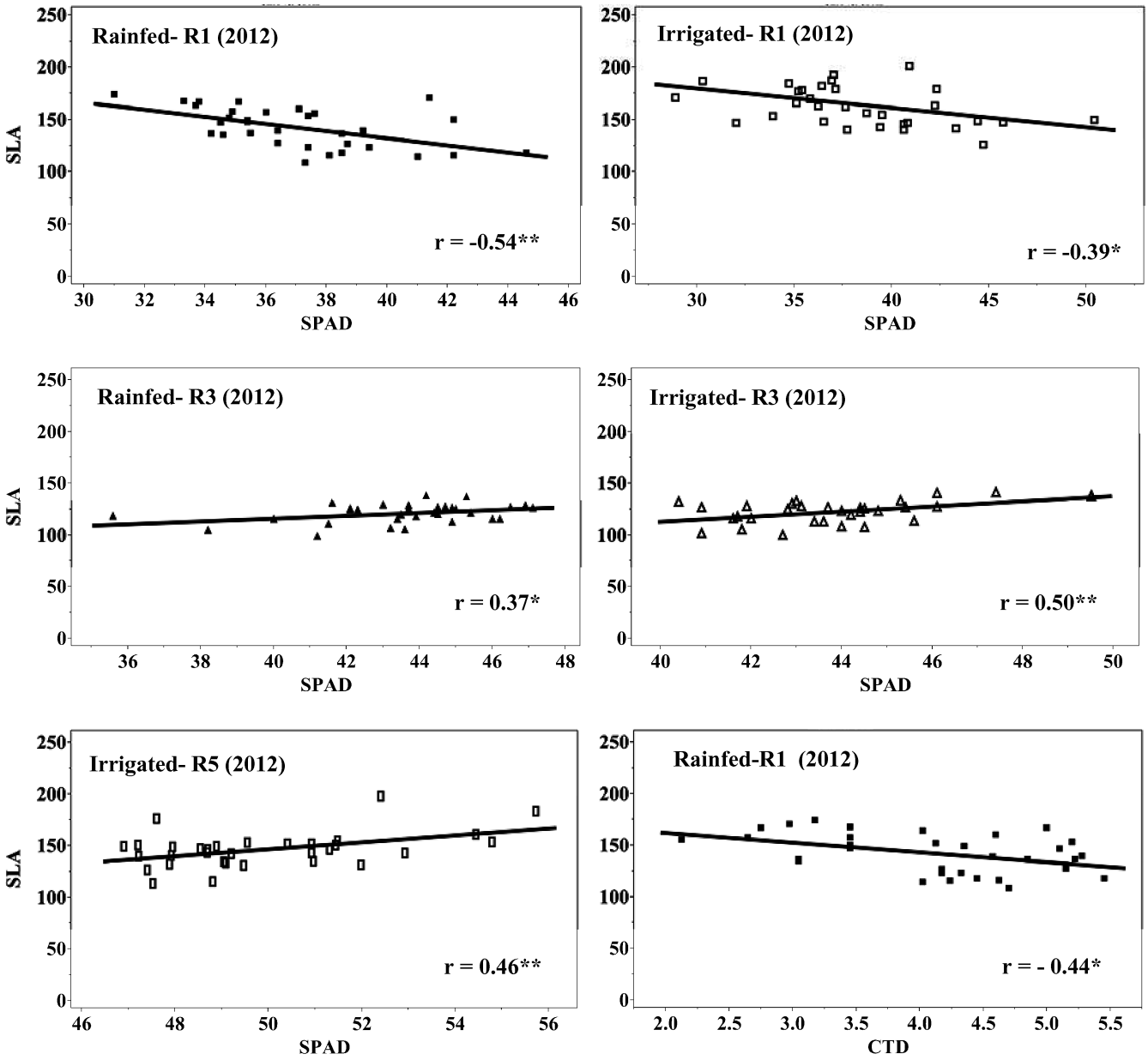

Fig. 2. Correlation of CTD, SPAD chlorophyll content, and SLA for eight virginia-type peanut genotypes at three growth stages (R1, beginning flower; R3, beginning pod; and R5, beginning seed), two water regimes (rainfed and irrigated) during field conditions in 2012.

N05006 were the best yielding lines under rainfed production in this study.

Canopy Temperature Depression. CTD has been widely used as a selection tool to screen germplasm for heat and drought tolerance in several crops (Amani et al., 1996; Balota et al., 2012a; Turner et al., 1986). The working principle of CTD is that under water stress, transpiration decreases due to stomata closure, but the tolerant genotypes tend to maintain higher transpiration rates, and therefore cooler canopies, than do the sensitive genotypes (Jackson, 1982; Turner et al., 1986). In the current study, no significant differences among genotypes were observed for CTD. Rainfed plants had on an average reduced CTD compared to irrigated plants (2.1 *vs.* 3.1 °C) in 2011, which was a dry year. In 2012, which was a wet year, irrigated plants were warmer (low CTD values) than rainfed plants (Table 5). This was probably due to excessive soil moisture in the irrigated plots that could have negatively affected the roots (Bradford and Hsiao, 1982; Jackson and Hall, 1987) resulting in reduced transpiration and therefore warmer plant canopies (reduced CTD). Canopy temperature depression was not correlated with pod yield in any water regime, growth stage, and year (Fig. 1). In 2012 under rainfed water regime, CTD measured early in the season

(stage R1) showed a negative correlation with SLA ($r=-0.44$, $p\leq0.05$). This suggests that reduction of SLA could be a physiological mechanism of peanut adaptation to rainfed production in sub-humid environments through better heat dissipation from thin leaves (higher SLA) when transpiration during the stress is low (Craufurd *et al.*, 1999), but this needs to be confirmed by future observations. Absence of genotypic variation and correlation with pod yield suggests that CTD approach is not suited for genotypic comparisons for yield and water deficit tolerance in peanut grown in sub-humid environments.

Specific Leaf Area and SPAD Chlorophyll Reading. In both years, growth stage had a significant effect on the SLA and SPAD chlorophyll readings with the smallest SLA values being recorded at R3 (beginning pod), and the greenest leaves at R5 (beginning seed) (Tables 6 to 8). Overall, leaves from rainfed plants had higher SLA than irrigated plant leaves in 2011; but in 2012, leaves from irrigated plants had higher SLA than rainfed plant leaves (Table 8). Also, SPAD chlorophyll reading was higher in rainfed than irrigated plants in 2011, and higher in irrigated than in rainfed plants in 2012. Many studies report a decrease of the SLA in response to water deficit (Arunyanark *et al.*, 2008; Upadhyaya, 2005), but others show an opposite trend (Wu *et al.*, 2008). In our study, the observed increase of SLA can only be related to response to stress in general, if considering that irrigated plots in 2012 experienced excessive moisture and that plants under this water regime were more stressed than those in rainfed plots. There was no relationship between SLA and the SPAD chlorophyll reading in 2011, but a significant positive correlation was observed at stages R3 in rainfed ($r = 0.37$, $p\leq0.05$) and irrigated ($r = 0.50$, $p\leq0.01$) plots, and R5 in irrigated ($r = 0.46$, $p\leq0.01$) plots in 2012 (Fig. 2). This can be explained by a considerable cloudiness in 2012, approximately 30% less incident radiation than in 2011, for which parallel increase of leaf area (high SLA) and chlorophyll content (better light capture) could have been necessary for increased photosynthesis and yield. There was no relationship between SLA and pod yield at any growth stage, water regime, and year. In 2012, SPAD chlorophyll readings at all three growth stages were significantly correlated to yield with coefficients of correlation ranging from 0.39 to 0.48 (Fig. 1). However, no relationship between yield and SPAD chlorophyll readings was detected in 2011.

In summary and based on these results, none of the physiological characteristics measured in this study were consistently associated with yield and genotypic tolerance to water deficit in sub-humid climates where water stress level may be too low in some years for systematic field selection. Even though genotypic variability existed for SLA and SPAD chlorophyll reading, confirming our earlier findings (Balota *et al.*, 2012), their relationship with pod yield did not exist (CTD and SLA) or was limited to only one year (SPAD chlorophyll reading). This suggests that additional screening methods should be considered in sub-humid climates such as use of rain exclusion shelters or greenhouse coupled with field tests to increase the amount of drought stress in years and at growth stages when natural stress is absent.

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Literature Cited

Amani, I., Fischer, R.A., and Reynolds, M.P. 1996. Canopy temperature depression association with yield of irrigated spring wheat cultivars in a hot climate. J. Agron. Crop Sci. 176:119-129.

Arunyanark, A., Jogloy, S., Akkasaeng, C., Vorasoot, N., Kesmala, T., Nageswara Rao, R.C., Wright, G.C., and Patanothai, A. 2008. Chlorophyll stability is an indicator of drought tolerance in peanut. J. Agron. Crop Sci. 194:113-125.

Ayeneh, A., van Ginkel, M., Reynolds, M.P., and Ammar, K. 2002. Comparison of leaf, spike, peduncle and canopy temperature depression in wheat under heat stress. Field Crops Res. 79:173-184.

Balota, M., Payne, W.A., Evett, S.R., and Peters, T.R. 2008. Morphological and physiological traits associated with canopy temperature depression in three closely related wheat lines. Crop Sci. 48:1897-1910.

Balota, M., Isleib, T.G., and Tallury, S. 2012a. Variability for drought related traits of virginia-type peanut cultivars and advanced breeding lines. Crop Sci. 52:2702-2713.

Balota, M., McGrath, S., Isleib, T.G., and Tallury, S. 2012b. Transpiration response to vapor pressure deficit in field grown peanut. Peanut Sci. 39:53-61.

Bell, M.J., Roy, R.C., Tollenaar, M., and Michaels, T.E. 1994. Importance of variation in chilling tolerance for peanut genotypic adaptation to cool, short-season environments. Crop Sci. 34:1030-1039.

Blum, A., Mayer, J., and Golan, G. 1988. The effect of grain number per ear (sink size) on source activity and its water-relations in wheat. J. Exp. Bot. 39:106-114.

Boote, K.J. 1982. Growth stages of peanut (*Arachis hypogaea* L.). Peanut Sci. 9:35-40.

Bradford, K.J. and Hsiao, T.C. 1982. Stomatal behavior and water relations of waterlogged tomato plants. Plant Physiol. 70:1508-1513.

Chapman, S.C. and Barreto, H.J. 1997. Using a chlorophyll meter to estimate specific leaf nitrogen of tropical maize during vegetative growth. Agron. J. 89:557-562.

Collaku, A. and Harrison, S.A. 2002. Losses in wheat due to waterlogging. Crop Sci. 42:444-450.

Craufurd, P.Q., Wheeler, T.R., Ellis, R.H., Summerfield, R.J., and Williams, J.H. 1999. Effect of temperature and water deficit on

water-use efficiency, carbon isotope discrimination, and specific leaf area in peanut. Crop Sci. 39:136-142.
FAOSTAT. 2013, Statistical Data Base. Food and Agricultural Organizations of the United Nations.
Isleib, T.G., Milla-Lewis, S.R., Pattee, H.E., Copeland, S.C., Zuleta, M.C., Shew, B.B., Hollowell, J.E., Sanders, T.H., Dean, L.O., Hendrix, K.W., Balota, M., and Chapin, J.W. 2011. Registration of 'Bailey' peanut. J. Plant Reg. 5:27-39. (doi:10.3198/jpr2009.12.0742crc)
Isleib, T.G., Rice, P.W., Mozingo, IIR.W., Copeland, S.C., Graeber, J.B., Pattee, H.E., Sanders, T.H., Mozingo, R.W., and Coker, D.L. 2006. Registration of 'Phillips' peanut. Crop Sci. 46:2308-2309.
Jackson, M.B. and Hall, K.C. 1987. Early stomatal closure in waterlogged pea plants is mediated by abscisic acid in the absence of foliar water deficits. Plant Cell Environ. 10:121-130.
Jackson, R.D. 1982. Canopy temperature and crop water stress. Adv. Irrig. 1:43-85.
Mozingo, R.W., Coffelt, T.A., Phipps, P.M., and Coker, D.L. 2006. Registration of 'CHAMPS' peanut. Crop Sci. 46:2711-2712.
Nageswara Rao, R.C., Talwar, H.S., and Wright, G.C. 2001. Rapid assessment of specific leaf area and leaf nitrogen in peanut (*Arachis hypogaea* L.) using a chlorophyll meter. J. Agron. Crop Sci. 186:175-182.
O'Toole, J.C., Turner, N.C., Namuco, O.P., Dingkuhn, M., and Gomez, K.A. 1984. Comparison of some crop water stress measurement methods. Crop Sci. 24:1121-1128.
Reynolds, M., Manes, Y., Izanloo, A., and Langridge, P. 2009. Phenotyping approaches for physiological breeding and gene discovery in wheat. Ann.Appl.Biol. 155:309-320.
Rorie, R.L., Purcell, L.C., Mozaffari, M., Karcher, D.E., King, C.A., Marsh, M.C., and Longer, D.E. 2011. Association of "greenness" in corn with yield and leaf nitrogen concentration. Agron. J. 103:529-535.
Rosas-Anderson, P.J. 2013. Peanut genetic variation for recovery from severe soil drying and low epidermal conductance as a drought tolerance trait. M.S. thesis. North Carolina State University, Raleigh.
SAS Institute Inc. 2012, SAS Institute Cary, NC, USA.
Sheshshayee, M.S., Bindumadhava, H., Rachaputi, N.R., Prasad, T.G., Udayakumar, M., Wright, G.C., and Nigam, S.N. 2006. Leaf chlorophyll concentration relates to transpiration efficiency in peanut. Ann. Appl. Biol. 148:7-15.
Songsri, P., Jogloy, S., Holbrook, C.C., Kesmala, T., Vorasoot, N., Akkasaeng, C., and Patanothai, A. 2009. Association of root, specific leaf area and SPAD chlorophyll meter reading to water use efficiency of peanut under different available soil water. Agr. Water Manage. 96:790-798.
Turner, N.C., O'Toole, J.C., Cruz, R.T., Namuco, O.S., and Ahmad, S. 1986. Responses of seven diverse rice cultivars to water deficits I. Stress development, canopy temperature, leaf rolling and growth. Field Crops Res. 13:257-271.
Upadhyaya, H.D. 2005. Variability for drought resistance related traits in the mini core collection of peanut. Crop Sci. 45:1432-1440.
Wu, F.Z., Bao, W.K., Li, F.L., and Wu, N. 2008. Effects of water stress and nitrogen supply on leaf gas exchange and fluorescence parameters of *Sophora davidii* seedlings. Photosynthetica 46: 40-48.