Journal of Plant Breeding and Crop Science Vol. 4(9), pp. 144-155, June 2012
Available online at http://www.academicjournals.org/JPBCS
DOI: 10.5897/JPBCS12.031
ISSN 2006-9758 ©2012 Academic Journals

Full Length Research Paper

Variation in carbon isotope ratio and its relation to other traits in peanut breeding lines and cultivars from U.S. trials

D. Rowland[1,2], N. Puppala[3], J. Beasley[4], Jr., M. Burow[5], D. Gorbet[6], D. Jordan[7], H. Melouk[8], C. Simpson[9], J. Bostick[10] and J. Ferrell[2]

[1]USDA-ARS, National Peanut Research Laboratory, Dawson, GA 39842, USA.
[2]Department of Agronomy, University of Florida, Gainesville, FL 32611, USA.
[3]New Mexico State University, Las Cruces, NM 88003, USA.
[4]University of Georgia, Tifton, GA 31793, USA.
[5]Texas A and M University, Lubbock, TX 79403, USA.
[6]North Florida Research and Education Center, Marianna, FL 32446, USA.
[7]NC State University, Raleigh, NC 27695, USA.
[8]USDA/ARS, Oklahoma State University, Stillwater, OK 74078, USA.
[9]C.E. Simpson, Texas A&M University, Stephenville, TX 76401, USA.
[10]Jim Bostick, Alabama Crop Improvement Association, P. O. Box 357, Headland, AL36345, USA.

Accepted 24 May, 2012

Water availability across U.S. peanut (*Arachis hypogaea* L.) producing areas is becoming threatened due to years of drought and competing urban demands. High water-use efficiency (WUE) has now become a priority in many peanut breeding programs. To support this effort, the variation in WUE, as measured by carbon isotope composition ($\delta^{13}C$), of up to 19 cultivars was evaluated in Alabama, Georgia, Florida, New Mexico, North Carolina, Oklahoma and Texas. Additionally, the variation in $\delta^{15}N$, percent carbon and nitrogen, SPAD chlorophyll content and specific leaf area corrected for solar radiation and VPD (SLA_{RV}) was determined. SLA_{RV} and SPAD chlorophyll were correlated with carbon isotope composition ($\delta^{13}C$) to determine if these inexpensive measurements could be used as selection tools for WUE in breeding programs. For genotypes measured at several sites simultaneously, genetic, environmental, and genotype X environment interactions were found to significantly affect most traits. Variation among genotypes grown at single sites was also found for measured traits. Genotypes were ranked within each site according to $\delta^{13}C$. Lastly, SLA_{RV}, SPAD, and $\delta^{13}C$ were correlated, but the significance and direction of the correlation was highly variable within regions and years making the use of these measurements for surrogates of WUE in U.S. breeding programs limited.

Key words: Water-use efficiency, carbon isotope, nitrogen isotope, drought.

INTRODUCTION

Drought and decreased water availability through urban use has affected almost every peanut (*Arachis hypogaea* L.) producing region in the U.S. over the last decade. In certain regions, the problem is reaching critical levels. For example in 2002, 54 counties in the North Carolina agriculture belt were declared disaster areas due to high crop losses during a severe growing season drought (Governor's office, U.S. Department of Agriculture). These high economic losses are compounded by increased aflatoxin risks in dryland peanut production, making growers increasingly dependent on irrigation to make economically viable yields. Irrigated peanut hectarage now comprises over 50% of all peanut

*Corresponding author. E-mail: dlrowland@ufl.edu. Tel: 352-273-3408.

production in the U.S. and can increase yields by up to 19% over dryland production (Lamb et al., 1997). Due to the often precarious balance between these increased yields and the high cost of irrigation equipment, maintenance, and fuel, it becomes necessary for a grower to maximize the efficiency of water application as much as possible. This production scenario makes the maximization of peanut water-use efficiency critical in U.S. peanut producing regions.

Water-use efficiency (WUE) is defined as the ratio of photosynthesis to transpiration and can be an important limitation to productivity under drought (Nageswara and Wright, 1994). However, increased WUE at the expense of yield has limited utility in agricultural systems. Peanut has the potential to have very high photosynthetic capacity accompanied by low stomatal conductance levels, translating into high WUE without sacrificing carbon assimilation and possibly yield (Wright et al., 1993). In addition, increased WUE can be attained by minimal to moderate reductions in stomatal conductance and transpiration that often do not cause a concomitant reduction in photosynthetic assimilation (Yoo et al., 2009), thus, incrementally increasing WUE without sacrificing assimilation and possibly production. These characteristics might allow the successful breeding of improved water-use efficient peanut genotypes while maintaining current production levels.

The problem with screening germplasm for WUE in a breeding program is the complex methods used for determining WUE directly (Wright et al., 1993). Weighing lysimeters and detailed measurements of water applied throughout the growing season are required in traditional procedures (Wright et al., 1988; Hatfield et al., 1989). However, researchers have found that the isotopic discrimination of ^{13}C that occurs during the photosynthetic pathway in C_3 plants (Farquhar et al., 1982; Farquhar and Richards, 1984) is well correlated with WUE in peanut and provides a long-term measurement of WUE across the season (Wright et al., 1993; Nageswara and Wright, 1994). The physiological principles behind this relationship occur due to the differential diffusivities across the stomatal aperture of CO_2 containing ^{12}C and ^{13}C, and the inherent discrimination against ^{13}C in favor of ^{12}C by Rubisco, the primary carbon fixation enzyme of photosynthesis (Farquhar et al., 1989). In the normal photosynthetic process, the isotopic composition of carbon fixed is heavily discriminatory of ^{13}C. However, for those plants with increased water-use efficiency, stomatal apertures may be decreased, causing increased assimilation of ^{13}C and thus, increasing the $^{13}C/^{12}C$ isotopic ratio in leaf tissue (Farquhar and Richards, 1984). Often the relationship of $^{13}C/^{12}C$ is examined by measuring the carbon isotopic discrimination (CID or Δ) of ^{13}C in leaf tissue (Farquhar et al., 1989). However, a direct measurement of the composition of $^{13}C/^{12}C$ ($\delta^{13}C$) rather than discrimination of ^{13}C (Δ) is also acceptable (Farquhar et al., 1989). In this case, $\delta^{13}C$ generally has a positive relationship with WUE such that high values of $\delta^{13}C$ represent high relative values of WUE.

Because of the high cost of stable isotope analyses, further research has examined the relationship between two easily and inexpensively measured characters, specific leaf area (SLA) and SPAD chlorophyll content, with carbon isotope discrimination (Nageswara and Wright, 1994; Nageswara et al., 1995; Lal et al., 2006; Sheshshayee et al., 2006). These relationships could provide useful, inexpensive, and easily utilized tools to screen large numbers of breeding lines simply by measuring leaf area and dry weight or SPAD reading. Water-use efficiency is positively correlated with $\delta^{13}C$ composition (that is, the higher the $\delta^{13}C$ composition, the higher the WUE), but negatively correlated with SLA such that thick leaves (low SLA) are predicted to have high WUE.

To study the relationship between $\delta^{13}C$ and SLA across disparate environments, researchers found that the correction of SLA (SLA_{RV}) for the total solar radiation incident on the crop (R) and the vapor pressure deficit (VPD) that prevailed one day prior to the collection of tissue for carbon isotope analysis improved the standardization of the SLA measurement in studies that were conducted across disparate regions (Nageswara et al., 2001). Further investigations have found a correlation between SLA_{RV} and SPAD chlorophyll content (Nageswara et al., 2001; Upadhyaya, 2005; Songsri et al., 2008, 2009); between $\delta^{13}C$ and SPAD directly (Sheshshayee et al., 2006); and between WUE and SPAD under a range of water availability (Songsri et al., 2009). These surrogate measurements have promise in breeding for increased WUE since SPAD, SLA, and carbon isotope discrimination have all been found to have additive genetic effects (Lal et al., 2006) and high heritability (Songsri et al., 2008).

As exciting as these results have been, many of these experiments documenting genotypic differences in $\delta^{13}C$ content and correlating $\delta^{13}C$ content and SLA or SPAD have been conducted in greenhouse environments utilizing pots or in field environments at single research sites, and usually involve extremely diverse germplasm (often interspecific comparisons) with genotypes not utilized in commercial U.S. peanut production. Therefore, genetic variation in WUE among commercially grown and often less genetically diverse peanut varieties is not known, nor the role that environment plays in changing the expression of these genetic differences. Furthermore, the relationship between carbon isotope discrimination, SLA and SPAD has not been documented in these conditions as well. Surveys of the genetic variation in WUE is needed for U.S. peanut genotypes and the direct relationship among $\delta^{13}C$, SLA_{RV} and SPAD chlorophyll content should be determined to provide information about the utility of these techniques for screening tools in U.S. breeding programs developing high water-use efficient peanut genotypes.

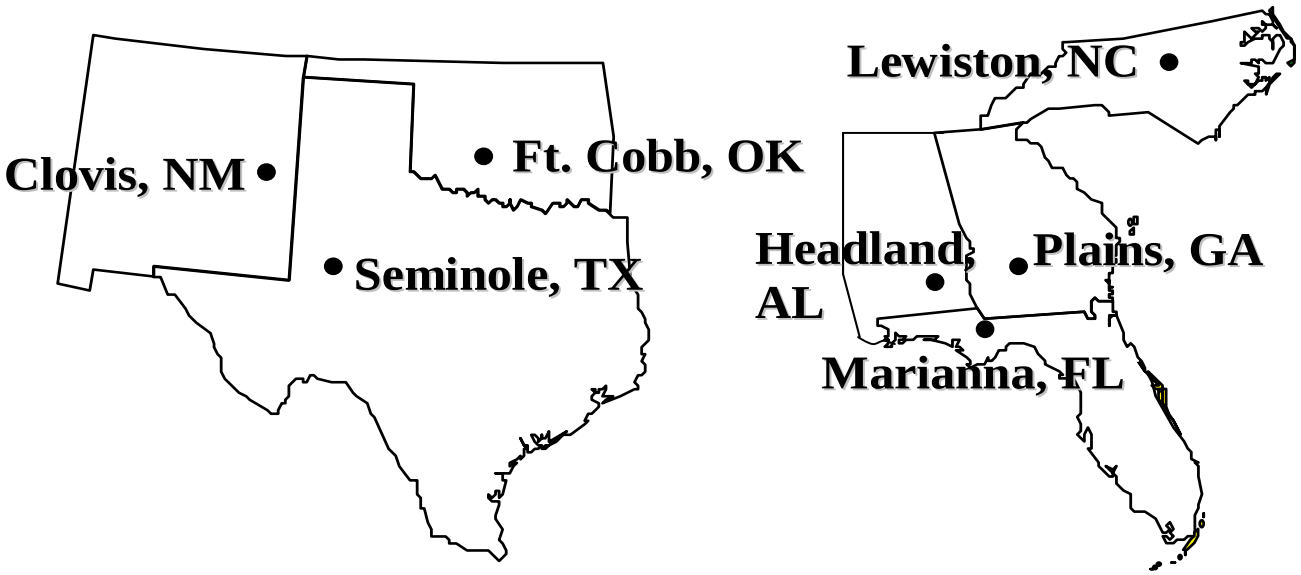

Figure 1. Sampling sites within the major peanut producing areas of the U.S. Several peanut genotypes at each site were sampled for $\delta^{13}C$, $\delta^{15}N$, SLARV, SPAD, percent carbon and percent nitrogen.

Water-use efficiency can also be affected by nitrogen fixation. The relationship is based on the dependence of both photosynthesis and stomatal conductance (determinants of WUE) on leaf nitrogen content (Guehl et al., 1998). Theoretical analyses and field data suggest that WUE may interact with nitrogen fixation and other nitrogen nutrition effects (Schulze et al., 1991; Guehl et al., 1998). The overriding pattern appears to be a positive correlation between $\delta^{13}C$ and $\delta^{15}N$, such that N_2 fixation (low $\delta^{15}N$ values) are associated with reduced water-use efficiency (more negative $\delta^{13}C$) (Schulze et al., 1991; Handley et al., 1994; Knight et al., 1993). Therefore, in studies examining $\delta^{13}C$ in a leguminous crop, it is important to likewise examine the relationship between $\delta^{13}C$ and $\delta^{15}N$ and the implications for nitrogen fixation.

This experiment was conducted in order to document genetic variation and the effect of environment on peanut WUE for U.S. grown genotypes and to quantify the relationship between $\delta^{13}C$, SLA_{RV}, SPAD, and $\delta^{15}N$ directly in these genotypes. The design implemented existing breeding trials across the U.S. peanut production areas, so there were two levels of comparison: a) across genotypes grown in several different regions simultaneously (a smaller subset of genotypes), and b) within individual sites comparing all genotypes being evaluated in the breeding trial at that site. The specific experimental objectives were: 1) to determine if genetic variability existed in WUE among several commonly grown U.S. peanut genotypes, 2) to determine if differing geographical regions affected the pattern of variability, and 3) to determine if WUE in U.S. peanut genotypes was directly correlated with SLA_{RV}, SPAD, and $\delta^{15}N$ measurements.

MATERIALS AND METHODS

Plant collection

During the 2001and 2002 growing seasons, tissue collections were made in existing peanut breeding trials across the U.S. peanut production region. The type of germplasm sampled at a given site depended on what genotypes had been included in each trial. In 2001, tissue was collected from six sites: 1) Plains, GA; 2) Marianna, FL; 3) Lewiston, NC; 4) Seminole, TX; 5) Clovis, NM; and 6) Ft. Cobb, OK. In 2002, all sites were sampled a second time and a site in Headland, AL was added for a total of seven sites (Figure 1). In each breeding trial, the peanut genotypes were grown in replicated trials used for breeding line evaluation in randomized block designs. All sites were managed under optimal conditions: using adequate irrigation to avoid drought stress; optimal soil nutrition (addition of N is sometimes necessary in western U.S. regions but typically not added in other regions); optimal disease management procedures; and using best management practices as dictated by regional recommendations. $\delta^{13}C$, $\delta^{15}N$, percent carbon, percent nitrogen, SLA_{RV}, and SPAD chlorophyll content were measured at each site and during both years. Three replications of each genotype were sampled, with leaf samples collected from six plants spaced along two rows per replication. Sampling was completed in a single day and within the morning hours (800 to 1200) at each site except Florida where sampling continued throughout the day.

In both years, peanut leaf tissue was collected approximately 90 days after planting (ranging from late July to mid-August, 2001). This phenological period is associated with the highest ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) levels and concomitantly the highest photosynthetic rates of the season. Sampling during this time period ensures varietal differences in photosynthesis (and therefore WUE) would be most evident (Nageswara and Wright, 1994; Nageswara et al., 1995). Tissue collection was standardized to second nodal apex leaves that had relatively no insect or disease damage. Standardizing to the second nodal leaf position has been shown to maximize the relationship between chlorophyll content and specific leaf area (Nageswara et

al., 2001). Tetrafoliate leaves were excised and chlorophyll content was measured using the Minolta SPAD chlorophyll meter directly after removal from the plant (Minolta Corp., Ramsey, N.J.). The SPAD chlorophyll meter measures absorbance by plant tissues of wavelengths in the visible spectrum and serves as a measure of the relative internal concentration of chlorophylls a and b. One SPAD chlorophyll reading was taken on each of the four leaflets, avoiding the midrib, and then averaged for one chlorophyll reading per plant to correct for possible non-homogeneous distribution of chlorophyll throughout the leaf (Monje and Bugbee, 1992). Tetrafoliate leaves were then placed on ice and refrigerated at 4°C until further analysis. Leaves were taken back to the laboratory and hydrated in distilled water for at least three hours prior to leaf area measurement in order to bring them all to a standardized turgor level (Nageswara et al., 2001). Leaflets were removed from each petiole and the leaf area of the four leaflets was measured with an LI-3000A leaf area meter (LI-COR Inc., Lincoln, NE) and summed to give total leaf area. Leaves were then oven dried at 60°C for 72 h and weighed. Specific leaf area (SLA) was calculated as the ratio of leaf area to leaf dry weight. Leaves were then fine ground using a Braun ® (model KSM2) coffee grinder and analyzed for carbon isotope composition ($\delta^{13}C$), $\delta^{15}N$, percent carbon (C), and percent nitrogen (N). Corrections for vapor pressure deficit (VPD) on SLA (SLA_{RV}) were applied following the method as described by Nageswara et al. (2001); but by replacing the measurement of "prevailing VPD" by logged VPD and incident radiation (R) measured on 15 min intervals using HOBO ® (Onset Computer Corporation, Bourne, MA) data loggers throughout the day prior to tissue sampling. VPD and R values were averaged between the hours of 9 A.M. and 4 P.M. (those hours that correspond with the majority of photosynthetic activity of the peanut plant), and the SLA correction from Nageswara et al. (2001) was applied as follows:

Corrected SLA (SLA_{RV}) = (SLA*VPD) / R

Where VPD and total solar radiation was in kPa and MJ/m^2, respectively. This formula was corrected from the original published value (Nageswara, personal communication).

In order to determine the isotopic composition in the peanut samples, leaf tissue was analyzed at the University of Arkansas Stable Isotope Laboratory in 2001 and at the Colorado Plateau Stable Isotope Laboratory, Department of Biological Sciences, Northern Arizona University in 2002. Samples of the ground leaves (2 mg, +/- 0.2 mg) were weighed, sealed in capsules and, along with standards, loaded into the elemental analyzer autosampler (a "Zero Blank "autosampler from Costech Analytical Technologies in Valencia, CA). Samples and standards were combusted in the elemental analyzer (Carlo Erba NC2500 elemental analyzer coupled with a Thermoquest Finnigan Delta plus isotope ratio mass spectrometer). Laboratory standards, which were calibrated against internationally distributed isotope standards, were analyzed at regular intervals throughout the sample runs. The resulting N_2 and CO_2 gases (along with isotopic reference gases for N_2 and CO_2) were admitted to the mass spectrometer through Finnigan's Conflo II interface. Data were collected and processed by Finnigan's Isodat software. Sample results were based on one analysis per sample ($\delta^{15}N$, $\delta^{13}C$, %N and %C were all determined with the same analysis). Isotope results were reported in delta notation vs. Air (for nitrogen) and vs. PDB (for carbon). Stable carbon isotope composition was expressed as $\delta^{13}C$ where $\delta^{13}C$ (‰) = [(R sample/R standard)-1] × 1000, and where R is the $^{13}C/^{12}C$ ratio.

Statistical analyses

Statistical analyses were performed using JMP SAS (SAS, 1997). Due to the use of existing breeder trial plots, the genotypes at each of the seven locations were not identical. Use of these plots led to different types and uneven numbers of genotypes at each site and an imbalanced design. To address this issue, two statistical analyses were run in order to address two separate objectives: 1) the comparison of similar genotypes grown across sites (a subset of all genotypes at a particular site) that could be used to examine genotype X environment interactions (*analysis 1*); and 2) the examination within sites to determine genetic variation in WUE within one environment (*analysis 2*). The genotypes measured in the first analysis included: Georgia Green, AT201, and C99R grown in Georgia and Florida; Gregory, NCV11, NC12C, Perry, and VA98R grown in Florida and North Carolina; and Flavor Runner 458, Georgia Green, and Tamrun96 grown in Oklahoma and Texas. For these regions, a factorial analysis of variance was run with year, site, genotype, replication, plant nested within replication, and the interactions between year/site, year/variety, and site/variety (genotype by environment interaction) as factors. Significant differences among varieties were determined using a Turkey's HSD multiple comparisons test. For the second analysis determining variation among peanut genotypes within a site, nested analysis of variance with genotype as the main effect and replication nested within genotype and plant nested within replication was performed within each of the sites and years individually. Differences among genotypes were determined using Turkey's HSD multiple comparisons test. Lastly, Pearson product-moment correlations were used to determine the relationship between $\delta^{13}C$, $\delta^{15}N$, SLA_{RV}, and SPAD chlorophyll. Correlations were run across all sites and genotypes and within sites, years, and peanut market types.

RESULTS

Genotype and environment effects on WUE: Analysis1

For the set of peanut genotypes grown simultaneously at different sites, the effects of year, site, genotype, and genotype X environment (G × E) interactions could be evaluated (Table 1). In all three regional comparisons (GA/FL, FL/NC, and OK/TX), there were significant differences between 2001 and 2002 for all measured leaf traits ($\delta^{13}C$, $\delta^{15}N$, Percent C, Percent N, SLA_{RV}, and SPAD), except for SPAD in GA and FL and percent nitrogen in OK and TX. A significant effect of site environment was most prevalent among the genotypes grown in GA and FL, where only $\delta^{15}N$ showed no differences among sites.

Site environment also affected genotypes grown in FL and NC where all traits except $\delta^{13}C$ and $\delta^{15}N$ were significantly different among sites; however, site environment was less important for genotypes grown in OK and TX, where only $\delta^{13}C$, SLA_{RV}, and SPAD were different among sites. When grown in GA and FL, Georgia Green, AT201, and C99R were significantly different from one another in all traits measured. Differences among genotypes were more subtle at the other two regions: 1) $\delta^{13}C$, $\delta^{15}N$, and SPAD differed among Gregory, NCV11, NC12C, Perry and VA98R; and $\delta^{13}C$, percent leaf nitrogen, and SPAD differed among Flavor Runner 458, Georgia Green, and Tamrun 96 (Table 1). A genotype × environment interaction (site × genotype factor) for the measured leaf traits was most prevalent for genotypes grown in GA and FL, where all

Table 1. Analysis of variance results for the effect of year, site, genotype and the interactions between year/site, year/genotype, and site/genotype on $\delta^{13}C$, $\delta^{15}N$, percent C, percent N, SLA_{RV}, and SPAD in leaf tissue. Values shown are F Ratios with associated p-values. The effects were analyzed in three groups of cultivars grown simultaneously at different sites: 1) Georgia Green, AT201, C99R measured in Georgia and Florida; 2) Gregory, NCV11, NC12C, Perry, VA98R measured in Florida and North Carolina; and 3) FR458, Georgia Green, Tamrun96 measured in Oklahoma and Texas. SLA_{RV} was corrected as follows: SLA_{RV} = (SLA *VPD)/R where VPD is vapor pressure deficit and R is total incident radiation as measured one day prior to leaf collection.

Factors	Traits						
	df	$\delta^{13}C$	$\delta^{15}N$	% C	% N	SLA_{RV}	SPAD
GA and FL							
Year	1	65.1**	114.7**	283.0**	18.4**	257.3**	1.5
Site	1	4.0 *	2.4	109.4**	179.8**	9.8**	12.7**
Genotype	2	21.4**	3.2 *	3.1 *	12.9**	15.7**	7.2**
Year*Site	1	13.8**	0.3	11.2**	2.7	17.4**	0.3
Year*Genotype	2	3.9 *	6.3**	1.7	2.4	0.7	0.7
Site*Genotype	2	3.4 *	8.1**	2.3	5.5 *	7.2**	2.0
Rep	2	2.2	1.1	7.7**	1.0	3.1 *	2.9
Plant (Rep)	15	0.9	0.8	0.7	0.8	1.0	0.4
FL and NC							
Year	1	13.8**	33.8**	515.7**	105.9**	1134.2**	15.4**
Site	1	0.0	1.9	77.0**	296.0**	769.1**	166.5**
Genotype	4	10.9**	3.3*	2.0	2.3	0.3	6.5**
Year*Site	1	1.8	69.0**	40.9**	45.8**	16.1**	17.4**
Year*Genotype	4	4.8**	1.2	3.6**	4.0**	2.4	4.1**
Site*Genotype	4	1.7	0.6	2.3	3.3*	3.0*	2.9*
Rep	2	1.1	4.5*	4.9**	1.9	0.6	3.8*
Plant (Rep)	15	0.8	0.8	1.3	1.0	1.7	0.6
OK and TX							
Year	1	59.0**	107.8**	298.6**	1.6	6.6*	21.3**
Site	1	5.7*	3.4	0.5	1.3	22.4**	49.8**
Genotype	2	27.4**	1.2	1.6	12.1**	1.0	9.8**
Year*Site	1	0.4	5.6*	2.9	68.0**	5.4*	47.6**
Year*Genotype	2	2.2	1.7	0.7	0.8	0.0	1.8
Site*Genotype	2	19.1**	1.4	3.7*	4.6*	0.5	1.7
Rep	2	0.2	1.8	4.9**	1.4	29.6**	0.9
Plant (Rep)	15	1.3	0.7	0.4	0.8	0.1	1.3

* Significant at $P< 0.05$; **Significant at $P< 0.01$.

traits except percent leaf carbon and SPAD showed G × E interactions. Genotypes grown in FL and NC had significant G × E interactions for percent leaf nitrogen, SLA_{RV}, and SPAD, while genotypes grown in OK and TX had significant G × E interactions for $\delta^{13}C$, percent leaf carbon, and percent leaf nitrogen (Table 1). The effect of individual factors on the means of measured leaf traits showed varying patterns (Table 2). Year (across sites) had a significant effect on $\delta^{13}C$ such that values were higher in 2002 than 2001 in the GA/FL and OK/TX comparisons; however, in the FL/NC comparison, genotypes at these sites had higher $\delta^{13}C$ values in 2001 than 2002. The values for $\delta^{15}N$ were consistently higher in 2002 than for 2001 for all three regional comparisons. Some broad differences among sites in trait means were also evident. SPAD chlorophyll content was significantly higher in North Carolina and in Georgia than for those same genotypes measured in Florida, while SLA_{RV} and SPAD were both lower in Oklahoma than Texas (Table 2). For those regional comparisons that showed a significant G × E interaction in $\delta^{13}C$, SLA_{RV}, and SPAD (Table 1), individual genotypes were tested by Tukey's HSD multiple comparisons test to determine which genotypes were significantly affected by site. In GA and FL, the cultivar C99R had significant differences in $\delta^{13}C$ and SLA_{RV}; in FL and NC, all genotypes showed

Table 2. Influence of year, site, and genotype on mean $\delta^{13}C$, $\delta^{15}N$, %C, %N, SLA_{RV}, and SPAD in peanut leaf tissue. The effects were analyzed in three groups of cultivars grown simultaneously at different sites: 1) Georgia Green, AT201, C99R measured in Georgia and Florida; 2) Gregory, NCV11, NC12C, Perry, VA98R measured in Florida and North Carolina; and 3) Flavor Runner 458, Georgia Green, Tamrun96 measured in Oklahoma and Texas. SLA_{RV} was corrected as follows: SLA_{RV}= (SLA *VPD)/R where VPD is vapor pressure deficit and R is total incident radiation as measured one day prior to leaf collection.

Year	Site	Genotype	Traits					
			$\delta^{13}C$	$\delta^{15}N$	% C	% N	SLA_{RV}	SPAD
GA and FL								
2001	FL	AT201	-26.6^{bcd}	0.39^{de}	43.6^{bc}	2.7^{e}	38.0^{f}	42.6^{a}
		C99R	-27.4^{e}	0.04^{e}	44.6^{ab}	3.7^{bcd}	49.3^{cde}	40.4^{ab}
		Georgia Green	-27.4^{e}	1.24^{abc}	43.6^{c}	2.6^{e}	44.2^{ef}	38.3^{b}
	GA	AT201	-27.2^{de}	0.72^{bcde}	45.2^{a}	4.1^{abc}	43.7^{ef}	42.0^{ab}
		C99R	-27.3^{de}	0.70^{cde}	44.7^{a}	4.2^{abc}	43.2^{ef}	43.4^{a}
		Georgia Green	-27.4^{e}	0.85^{bcd}	44.6^{ab}	4.1^{abc}	47.8^{de}	40.5^{ab}
2002	FL	AT201	-26.2^{abc}	1.77^{a}	41.3^{d}	2.9^{e}	58.8^{b}	41.8^{ab}
		C99R	-26.8^{cde}	1.40^{abc}	41.6^{d}	3.6^{cd}	68.2^{a}	40.7^{ab}
		Georgia Green	-27.2^{de}	1.72^{a}	41.3^{d}	3.3^{de}	67.8^{a}	39.8^{ab}
	GA	AT201	-25.8^{a}	1.74^{a}	42.8^{c}	4.4^{ab}	54.0^{bcd}	43.2^{a}
		C99R	-25.9^{ab}	1.97^{a}	43.4^{c}	4.8^{a}	56.8^{bc}	43.5^{a}
		Georgia Green	-26.8^{cde}	1.47^{ab}	43.3^{c}	4.7^{a}	61.2^{ab}	41.9^{ab}
FL and NC								
2001	FL	Gregory	-26.9^{abcd}	1.03^{abcd}	43.7^{abc}	2.3^{g}	34.2^{d}	39.9^{ghi}
		NC12C	-27.2^{cd}	0.21^{d}	44.0^{ab}	2.8d^{efg}	37.6^{d}	40.3^{fghi}
		NCV11	-26.1^{a}	0.83^{bcd}	43.4^{bcd}	2.4^{fg}	34.4^{d}	38.8^{hi}
		Perry	-27.0^{bcd}	0.15^{d}	44.1^{ab}	2.8^{defg}	37.9^{d}	42.0^{defghi}
		VA98R	-26.7^{abcd}	0.40^{cd}	43.3^{bcd}	2.6^{efg}	36.5^{d}	40.8^{efghi}
	NC	Gregory	-26.6^{abcd}	1.84^{ab}	43.8^{ab}	3.1^{de}	50.4^{c}	43.8^{cdefg}
		NC12C	-27.1^{bcd}	1.89^{abcd}	44.2^{ab}	3.1^{de}	53.2^{c}	42.0^{defghi}
		NCV11	-26.4^{ab}	1.32^{abc}	44.7^{a}	3.4^{cd}	52.4^{c}	46.0^{abcd}
		Perry	-26.8^{abcd}	1.45^{abc}	43.6^{abc}	3.1^{de}	51.4^{c}	42.3^{defgh}
		VA98R	-26.5^{abc}	1.43^{abc}	43.4^{bcd}	3.1^{de}	53.6bc	43.9^{cdefg}
2002	FL	Gregory	-27.2^{d}	2.01^{a}	40.6^{g}	2.9^{def}	61.0^{b}	40.9^{efghi}
		NC12C	-27.2^{cd}	1.43^{abc}	40.4^{g}	2.7^{efg}	53.5^{bc}	39.5^{hi}
		NCV11	-26.6^{abcd}	1.80^{ab}	41.1^{fg}	2.9^{def}	54.5^{bc}	42.7^{defgh}
		Perry	-26.7^{abcd}	2.10^{a}	40.6^{g}	2.6^{efg}	55.6^{bc}	38.0^{i}
		VA98R	-27.0^{bcd}	2.02^{a}	40.9^{fg}	2.7^{efg}	56.5^{bc}	40.2^{ghi}
	NC	Gregory	-27.2^{d}	1.34^{abc}	42.5^{cde}	4.0^{abc}	74.9^{a}	48.5^{a}
		NC12C	-27.1^{cd}	1.21^{abcd}	42.1^{ef}	3.8^{bc}	77.8^{a}	44.5^{abcde}
		NCV11	-27.0^{bcd}	1.11^{abcd}	42.5^{cde}	4.6^{a}	80.9^{a}	48.4^{ab}
		Perry	-26.7^{abcd}	1.18^{abcd}	41.9^{ef}	4.2^{ab}	80.0^{a}	44.3^{bcdef}
		VA98R	-27.0^{bcd}	1.20^{abcd}	42.4d^{e}	4.0^{ab}	74.9a	47.2^{abc}
OK and TX								
2001	OK	FlavRunner458	-25.6^{bc}	2.10^{bc}	43.7^{b}	3.4^{bcde}	41.5^{b}	41.7^{bcd}
		Georgia Green	-26.0^{bcd}	2.26^{bc}	44.0^{ab}	3.7^{ab}	42.9^{b}	44.0^{abc}
		Tamrun96	-26.3^{cde}	2.13^{bc}	45.1^{a}	3.7^{abc}	45.5^{ab}	43.5^{abc}
	TX	FlavRunner458	-25.8^{bcd}	2.41^{bc}	44.3^{ab}	2.8^{f}	62.9^{ab}	40.3^{cde}
		Georgia Green	-26.7^{e}	1.92^{c}	44.2^{ab}	3.5^{bcde}	72.4^{a}	44.9^{ab}
		Tamrun96	-25.8^{bcd}	1.94^{c}	43.8^{b}	3.1^{ef}	62.7^{ab}	44.3^{abc}

Table 2. Cont.

2002	OK	FlavRunner458	-24.9^{a}	3.17^{ab}	41.2^{c}	3.1e^{f}	40.9^{b}	35.4^{f}
		Georgia Green	-25.5^{ab}	3.89^{a}	41.4^{c}	3.1^{def}	44.9^{ab}	38.0^{def}
		Tamrun96	-25.4^{ab}	3.00^{abc}	41.4^{c}	3.1^{cdef}	41.8b	37.1^{ef}
	TX	FlavRunner458	-25.3^{ab}	3.87^{a}	41.5^{c}	3.5^{bcde}	47.9^{ab}	43.4^{abc}
		Georgia Green	-26.3^{de}	4.10^{a}	41.9^{c}	4.1^{a}	54.7^{ab}	43.0^{abc}
		Tamrun96	-24.9^{a}	3.95^{a}	41.7^{c}	3.6^{abcd}	48.2^{ab}	46.8^{a}

† For a given peanut type, means within columns followed by the same letter are not significantly different according to Turkey's HSD multi-comparisons test (α = 0.05).

Table 3. Analysis of variance results by year for variation among genotypes in $\delta^{13}C$, $\delta^{15}N$, **%**C, **%**N, SLA_{RV}, and SPAD chlorophyll content within individual sites and years. Mean values reported are averaged across all genotypes grown at that site and their ranking for $\delta^{13}C$ can be seen in Table 4. Symbols next to trait means represent significant differences among genotypes grown at that site. SLA_{RV} was corrected as follows: SLA_{RV} = (SLA *VPD)/R where VPD is vapor pressure deficit and R is incident radiation as measured one day prior to leaf collection.

Year	Site	Trait					
		$\delta^{13}C$	**$\delta^{15}N$**	**% C**	**% N**	**SLA_{RV}**	**SPAD**
2001	FL	-27.00***	0.45***	44.0***	2.9***	41.2***	40.6***
	GA	-27.27	0.90*	44.6**	3.9***	43.2***	41.7
	NC	-26.70***	1.49	44.1*	3.3***	54.6***	43.6***
	NM	-25.46***	2.48	43.8***	3.5***	26.4	35.9**
	OK	-26.33***	2.05**	44.4***	3.8***	47.1***	41.7***
	TX	-26.09***	2.25**	43.9***	3.1***	64.8***	42.4*
2002	AL	-25.58*	1.66	41.3*	2.8***	36.3***	37.4**
	FL	-26.67***	1.80***	41.0***	2.9***	59.5***	39.9***
	GA	-26.19***	1.73	43.2**	4.7	57.3**	43.0*
	NC	-27.16***	1.24*	42.1***	4.0***	79.0***	46.2***
	NM	-25.54***	4.63***	41.9**	2.8***	45.9***	35.0***
	OK	-25.32*	3.15***	41.3	3.1	42.3	36.8
	TX	-25.76***	4.29*	41.2***	3.4***	49.4***	40.1***

* Significant at *P*< 0.05; **Significant at *P*< 0.01; *** Significant at *P*< 0.001.

differences among sites in SLA_{RV} and SPAD; and in OK and TX, Georgia Green and Tamrun 96 showed differences among sites in $\delta^{13}C$ (Table 2).

Genetic variation within sites: Analysis 2

Because several peanut genotypes were grown at single sites only, it was important to evaluate differences among genotypes within sites individually in order to determine what, if any, genetic variation existed. There was significant variation in $\delta^{13}C$, $\delta^{15}N$, percent carbon, percent nitrogen, SLA_{RV}, and SPAD chlorophyll content in both 2001 and 2002 among those genotypes grown within single sites, indicating the existence of genetic variation for peanut WUE and leaf morphological characteristics (Table 3). Within sites, genotypes showed significant variation for $\delta^{13}C$ except those grown in Georgia in 2001. The highest $\delta^{13}C$ values were found in New Mexico in 2001 and in Oklahoma in 2002. The lowest $\delta^{13}C$ values were found in Georgia in 2001 and in North Carolina in 2002. There appeared to be a demarcation between values of $\delta^{15}N$ for the southeastern vs. southwestern states with the lowest values of $\delta^{15}N$ found in Alabama, Florida, Georgia, and North Carolina in both 2001 and 2002 (Table 3). Only Florida, Oklahoma, and Texas showed significant differences among genotypes in $\delta^{15}N$ for both years, with New Mexico having significant variation in 2002. Percent C of leaf tissue appeared to

Table 4. Ranking by $\delta^{13}C$ of peanut genotypes at each of seven sites throughout the major U.S. peanut production regions in 2001 and 2002. Genotypes are listed in order of $\delta^{13}C$ value; therefore, those listed first have higher $\delta^{13}C$ and higher WUE.

Year	Genotype ranks by site						
	AL	GA	FL	NC	NM	OK	TX
2001	Not measured	GAHiOL[a]†	NCV11[a]	NCV11[a]	GARed[a]	FR458[a]‡	Florunner[a]
		AT201[a]	Florunner[ab]	VA98R[ab]	GAValencia[b]	Florunner[a-c]	Tamrun96[a]
		C99R[a]	Tamrun96[a-c]	Gregory[ab]	Sunland[b]	GAGreen[a-d]	FR458[a]
		GAGreen[a]	AT201[a-d]	GAGreen[ab]	ValenciaC[b]	TX977006[c-e]	TX977006[a]
			GAHiOL[a-e]	VAC92R[abc]	ValenciaA[b]	Tamrun96[c-e]	TX977053[a]
			VA98R[a-f]	Perry[bc]	GT101[b]	TX977053[d-f]	Tamspan90 [b]
			Gregory[a-g]	NC12C[c]	GT102[b]	Tamspan90[d-f]	TX962120[b]
			VAC92R[a-g]			TX962120[fg]	GAGreen[b]
			Perry[b-g]			ValenciaA[g]	
			Carver[b-g]				
			AndruII[c-g]				
			NC12C[c-g]				
			ANorden[d-g]				
			Virugard[d-g]				
			GAGreen[e-g]				
			DP-1[e-g]				
			C99R[fg]				
			Hull[g]				
			UF00324[g]				
2002	Florunner[a]	AT201[a]	Tamrun96[a]	Perry[a]	FR458[a]	FR458[a]	Tamrun96[a]
	VA98R[a]	C99R[a]	Florunner[a]	NCV11[ab]	Florunner[ab]	Tamrun96[a]	FR458[a]
	NCV11[ab]	GAGreen[b]	AT201[ab]	VA98R[ab]	Tamrun96[ab]	Florunner[a]	Florunner[a]
	Gregory[ab]		NCV11[bc]	NC12C[b]	GARed[a-c]	GAGreen[a]	ValenciaA[b]
	AT201[ab]		Perry[bc]	Gregory[b]	GAValencia[bc]		GAGreen[b]
	C99R[ab]		C99R[bc]	AT201[b]	GAGreen[bc]		ValenciaC[b]
	GAGreen[b]‡		Carver[bc]	GAGreen[c]	Tamspan90[bc]		
			VA98R[bc]		GT101[bc]		
			NC12C[c]		ValenciaA[bc]		
			GAGreen[c]		ValenciaC[c]		
			ANorden[c]		GT102[c]		
			Gregory[c]				

† For a given year, means within columns followed by the same letter are not significantly different according to Turkey's HSD multi-comparisons test (α = 0.05); ‡ FR458 = Flavor Runner 458, GAGreen = Georgia Green.

vary little among sites with a range of 43.8 (New Mexico) to 44.6 (Georgia) in 2001 and 41.0 (Florida) to 43.2 (Georgia) in 2002. Georgia had the highest percent N than the other sites for both years (Table 3). Significant genotypic differences existed for SLA_{RV} and SPAD at all sites and years with the following exceptions: for New Mexico in 2001 and for Oklahoma in 2002 for SLA_{RV}, and for Georgia in 2001 and Oklahoma in 2002 for SPAD. The highest SLA_{RV} values were found in Texas in 2001 and North Carolina in 2002; while SPAD values were consistently lowest in New Mexico (35.9 in 2001 and 35.0 in 2002) and highest in North Carolina (43.6 in 2001 and 46.2 in 2002) (Table 3).

Genotypes were ranked according to $\delta^{13}C$ value, and due to the positive correlation between $\delta^{13}C$ and WUE in peanut, it can be assumed that lower (more negative) values of $\delta^{13}C$ indicate low WUE for the peanut genotypes measured in this study (Table 4). Ranking of cultivars did not seem to fall within growth habit types; runner types and Virginia types were interspersed among high, medium, and low WUE values within a single site. By examining the ranking of those cultivars that were grown simultaneously at more than one site, the influence of both genetic and environmental effects on WUE were apparent. Consistency of rank for a cultivar grown across sites indicated an apparent genetic control over $\delta^{13}C$. For

Table 5. Pearson product-moment correlations of $\delta^{13}C$, SLA_{RV}, SPAD chlorophyll content, $\delta^{15}N$, and percent nitrogen within seven sites across all genotypes within a site. SLA_{RV} was corrected as follows: SLA_{RV} = (SLA *VPD)/R where VPD is vapor pressure deficit and R is incident radiation as measured one day prior to leaf collection.

Site	Year	Pearson correlation value					
		$\delta^{13}C \times SLA_{RV}$	$\delta^{13}C \times$ SPAD	$SLA_{RV} \times$ SPAD	$\delta^{13}C \times \delta^{15}N$	$\delta^{15}N \times$ % N	$\delta^{13}C \times$ % N
ALA	2001	NA	NA†	NA	NA	NA	NA
	2002	-0.55**	NS‡	NS	NS	-0.36**	-0.55**
FLA	2001	-0.63**	-0.26**	-0.12*	0.29**	-0.48**	-0.63**
	2002	-0.55**	-0.47**	0.26**	0.47**	-0.50**	-0.63**
GA	2001	-0.51**	NS	-0.26*	NS	-0.49**	-0.44**
	2002	-0.54**	NS	NS	NS	NS	-0.29 *
NC	2001	-0.19 *	NS	NS	NS	-0.21 *	-0.24 **
	2002	NS	NS	NS	-0.23**	NS	0.27 **
NM	2001	-0.18*	0.28**	-0.49**	NS	-0.37**	NS
	2002	-0.37**	0.18**	-0.36**	NS	NS	NS
OK	2001	-0.57**	0.34**	-0.64**	NS	NS	-0.22 *
	2002	-0.47**	-0.42**	NS	NS	NS	NS
TX	2001	-0.38**	0.16*	NS	-0.24*	-0.45**	NS
	2002	NS	0.56**	NS	NS	-0.42**	NS

* Significant at $P< 0.05$; **Significant at $P< 0.01$; † No data available for this year; ‡ Correlation not significant.

example, Florunner and Tamrun96 (except Oklahoma in 2001) consistently had higher WUE than many cultivars at almost all sites indicating WUE may be primarily under genetic control for these cultivars.

Correlation of $\delta^{13}C$, SLA_{RV}, SPAD chlorophyll content, $\delta^{15}N$, and percent N

When pooling the data across all sites and genotypes within years, there were significant correlations of $\delta^{13}C$ with SLA_{RV} (2001: Pearson Correlation Value [PCV] = -0.28, p-value = 0.0001; 2002:PCV = -0.44, p-value = 0.0001), $\delta^{13}C$ and SPAD (2001:PCV = -0.12, p-value = 0.0001; 2002:PCV = -0.29, p-value = 0.0001), and SLA_{RV} and SPAD (2001:PCV = 0.17, p-value = 0.0001; 2002:PCV = 0.28, p-value = 0.0001). However, the strength of the correlations between these traits was affected by the environment and genotype in which the trait was measured. When examining the correlation within single sites, only the correlation between $\delta^{13}C$ and SLA_{RV} shows a consistent significant negative relationship for every site and year except North Carolina and Texas in 2002 (Table 5). This negative relationship showed that thicker leaves (lower SLA_{RV}) had higher relative $\delta^{13}C$ levels and increased water-use efficiency. The correlations between $\delta^{13}C$ and SPAD and between SLA_{RV} and SPAD are less consistent. There was no significant relationship between $\delta^{13}C$ and SPAD for Alabama, Georgia, and North Carolina for both years, while the correlation at the other sites was inconsistent both with negative patterns (Florida 2001, 2002; Oklahoma 2002) and positive patterns (New Mexico, 2001, 2002; Oklahoma, 2001, 2002; Texas 2001, 2002, 2003). The relationship between SLA_{RV} and SPAD was also inconsistent and oftentimes insignificant (Alabama 2002; Georgia, 2002; North Carolina, 2001, 2002) (Table 5).

The correlation of $\delta^{13}C$ with $\delta^{15}N$ and percent N, and the correlation of δ^{15} N with percent N showed variability among sites as well (Table 5). The correlation between $\delta^{13}C$ and $\delta^{15}N$ was non-existent at all sites except Florida in 2001 and 2002, North Carolina in 2002, and Texas in 2001. However, the direction of the relationship was inconsistent, with positive correlations in Florida and negative correlations in North Carolina and Texas. In 2001, all of the sites measured except Oklahoma had a significant negative correlation between $\delta^{15}N$ and percent N, showing the lower the $\delta^{15}N$ content (higher relative nitrogen fixation), the higher the tissue N. However, in 2002 this relationship was only significant for three sites: Alabama, Florida, and Texas. Similarly, $\delta^{13}C$ had a negative correlation with percent N for both years in Florida, Georgia, and North Carolina (Oklahoma, 2001; Alabama, 2002).

DISCUSSION

This study was successful in documenting the existence of genetic variation among commercially grown peanut genotypes in the U.S. for $\delta^{13}C$ (and thus WUE), $\delta^{15}N$, percent carbon, percent nitrogen, SLA_{RV}, and SPAD chlorophyll content, but environment played a significant

role in the genetic expression of these traits. The range of variability for $\delta^{13}C$ found in this study is similar to ranges found for other peanut cultivars, typically $\delta^{13}C$ = -28.0 and -26.8, as was found for an even more disparate group of peanut cultivars than was used in this study (Hubick et al., 1988). We expected variability actually to be lower than other studies because we chose the most prevalent germplasm commercially available which likely represented a very narrow range of genetic variability for WUE in these peanut cultivars. Although, the number of genotypes that were grown simultaneously at different sites limited the ability to determine G × E interactions for these traits, the existence of genetic, environmental and G × E interactions was evident. The existence of variation among different genotypes when grown within single sites also shows the genetic control over WUE in the peanut genotypes measured and supports previous work that documented genotypic variation in $\delta^{13}C$ and WUE for peanut in greenhouse (Hubick et al., 1986) and field conditions (Nageswara et al., 1993).

In peanut, these genetic differences in WUE appear to be due to photosynthetic capacity and not stomatal factors (Nageswara et al., 1995). Variability in WUE in plants can be due to stomatal diffusive properties (conductance types) or intrinsic photosynthetic capacity (capacitance types) (Udayakumar et al., 1998). The majority of plants evolved to use the former strategy where WUE is maximized by reducing transpiration (Udayakumar et al., 1998). In conductance types, high WUE is at the expense of dry matter production and yield. Peanut, however, has been shown to be a capacitance type where photosynthetic capacity and even rubisco levels per leaf area are the major factors contributing to variation in WUE (Nageswara et al., 1995; Nageswara et al., 2001). Therefore, selection for high WUE in peanut has the potential of being accompanied by high growth rates and yield (Udayakumar et al., 1998). This study has documented the potential genetic variation in U.S. peanut genotypes that can be utilized in breeding programs aimed at maximizing WUE.

The existence of G X E interactions for genotypes grown simultaneously at different sites (GA/FL, FL/NC, and OK/TX) has shown that both genetics and environment play a strong role in the expression of many of the measured leaf traits. A few previous studies in peanut documented that variation in $\delta^{13}C$ was influenced by location and genotype (Hubick, 1990; Nageswara and Wright, 1994; Brown and Byrd, 1996), but evidence of G × E interactions for WUE is scarce because most surveys of G × E interactions for $\delta^{13}C$ and thus WUE are done in limited numbers of environments. The current study was conducted across very diverse environments and regions, possibly making the expression of G × E more apparent. While genotypes compared in GA and FL and those in OK and TX showed strong G × E interactions for $\delta^{13}C$, the case is different for genotypes grown in FL and NC. The latter varieties were primarily Virginia genotypes, so that it appears that $\delta^{13}C$ and thus WUE may be more strongly genetically controlled in this market type. This is in agreement with Wright et al. (1988) who found a strong genetic control over $\delta^{13}C$ with little effect of the environment in four Virginia genotypes when grown either in open or closed canopies. Some inherent differences between Virginia and runner peanuts have been documented in the past for traits that may affect WUE, including different peak daily water requirements between runner and Virginia types – 85 days for runners and 100 days for Virginia (Stansell et al., 1976). The ranking of genotypes for $\delta^{13}C$ within single sites shows no real consistency across sites for runner or Virginia genotypes, probably because of the large differences in the numbers of genotypes measured at each site. One runner genotype, Georgia Green, showed a large impact of the environment on its genetic expression of $\delta^{13}C$ content, and because it was included in most breeding trials, this interaction could be examined across very diverse climates. This cultivar showed a consistent lower level of $\delta^{13}C$ when grown under the similar climates and environmental conditions of Florida and Georgia, with increased $\delta^{13}C$ when grown in Oklahoma and Texas. Therefore, this genotype appears to be more water-use efficient in arid environments.

Genetic variation in $\delta^{15}N$ was apparent in this study, but this trait was also significantly affected by the environment. The natural $\delta^{15}N$ isotopic composition of leaf tissue has been purported to be an indicator of nitrogen fixation and has been tested extensively for identifying nitrogen-fixing plants in ecosystems (Delwiche et al., 1979; Virginia and Delwiche, 1982). It can be inferred that the lower the $\delta^{15}N$ signal, the greater the rate of nitrogen fixation. The relationship is based on the fact that nitrogen gas has a lower $\delta^{15}N$ than nitrogen from the soil, so that plants that have a high nitrogen fixation rate will have lower $\delta^{15}N$ in their tissues than plants that rely more on soil nitrogen (Sprent et al., 1996; Wanek and Arndt, 2002). Nitrogen fixation rates have been estimated in peanut from tissue $\delta^{15}N$ (Bell and Wright, 1994). The results in this study suggest a distinction in nitrogen fixation rates between southeastern, humid sites and southwestern, arid sites. In both years, $\delta^{15}N$ leaf tissue contents were lower in southeastern states than their arid counterparts, indicating a higher rate of nitrogen fixation. This may, in part, be linked to the additional nitrogen typically added to southwestern U.S. breeding plots which may have been absent in southeastern breeding trials. The additional nitrogen may interfere with nodulation and thus lower nitrogen fixation rates in these southwestern plots.

The relationship between $\delta^{13}C$ and $\delta^{15}N$ was either inconsistent or nonexistent at almost every site, with the exception of Florida. Very few studies have related $\delta^{15}N$ with $\delta^{13}C$, and those that have show the relationship to be affected by environment or nonexistent (Guehl et al.,

1998).The results in this study showed that site and year had significant effects on the strength of the relationship between $\delta^{13}C$ and $\delta^{15}N$. The correlation between the two traits in Florida was positive, such that N_2 fixation (low $\delta^{15}N$ values) was associated with reduced water-use efficiency (more negative $\delta^{13}C$) (Schulze et al., 1991; Handley et al., 1994; Knight et al., 1993). However, the correlation was negative in North Carolina in 2002 and in Texas in 2001. In Florida, the positive correlations were found for the genotypes GAHiOL, Perry, Hull, VAC92R, and NCV11 (data not shown) indicating that in some high oleic or Virginia type peanuts, nitrogen fixation is accompanied by lowered WUE. However, these results are less than definitive. The correlation with $\delta^{15}N$ and percent nitrogen are more consistent. In 2001, the negative correlation between the two traits at every site except Oklahoma follows the assumption that high nitrogen fixation leads to high N contents (Virginia and Delwiche, 1982; Sprent et al., 1996).

One primary objective of this study was to examine the relationship between $\delta^{13}C$ and other more easily measured leaf traits. The results generally support the correlation between $\delta^{13}C$ and SLA (SLA_{RV} in the current study) found previously (Wright et al., 1993; Nageswara and Wright, 1994) but the correlation between SLA and SPAD noted in other studies (Nageswara et al., 2001; Upadhyaya, 2005; Songsri et al., 2008, 2009) was not evident in most sites in the current study. The physiological basis behind these relationships has been illustrated by the correlation of photosynthesis and leaf thickness in many crops including alfalfa, soybean, oats, and chickpea, suggesting that thicker leaves have a higher density of chlorophyll per unit leaf area giving them higher assimilation rates than thinner leaves (Nageswara and Wright, 1994; Nageswara et al., 1995; Craufurd et al., 1999). However, the disparity of the strength of the correlations among characteristics measured in this current study is troublesome and may be due to the fact that the relationship between $\delta^{13}C$ (or WUE) and SLA_{RV} is highly dependent on sampling procedure and environment (Nageswara et al., 2001; Songsri et al., 2009). Even though careful, standardized tissue collection procedures were followed in this study, the correlations among $\delta^{13}C$, SLA_{RV}, and SPAD differed in strength within sites and years (Table 5). This supports previous studies documenting a strong influence of the environment on SLA (Nageswara and Wright, 1994; Upadhyaya, 2005; Songsri et al., 2009) and may limit the utility of using SLA_{RV} as a screening tool in U.S. breeding programs across regions. Selection for SLA_{RV} would be based on phenotypically plastic responses to a given set of environmental conditions within a breeding region, thereby making the breeding stock appropriate only for very specific growing areas. Even more promising as an economical alternative for screening large numbers of germplasm for WUE was the SPAD chlorophyll meter (Nageswara et al., 2001; Sheshshayee et al., 2006).

However, in this study, the direct correlation of SPAD chlorophyll content with $\delta^{13}C$ level showed no consistent relationship as was found in a previous study with peanut grown in pots (Sheshshayee et al., 2006). This is unfortunate because SPAD chlorophyll content is less likely to be affected by environment (Upadhyaya, 2005) than SLA_{RV} and so would be more applicable in regional studies across diverse environments.

Conclusions

This study has documented variation in $\delta^{13}C$ (and thus, WUE) among commercially grown U.S. peanut genotype that has the potential to be utilized in breeding programs aimed at maximizing WUE. The utility of using easily measured and inexpensive traits such as SLA_{RV} or SPAD as correlates of $\delta^{13}C$ appears to be limited for these U.S. peanut genotypes when evaluated across diverse regions. There is a need for breeders and physiologists to collaborate more extensively in order to provide better knowledge of the physiological basis behind the performance of genotypes in different environments and speed up the development of superior genotypes (Wright et al., 1996). Currently, grown peanut genotypes and developing cultivars need to be assessed for WUE not only across different growing regions, but also different growing conditions. This may allow breeders to tailor varieties for production management methods and environmental conditions and to make better-informed cultivar recommendations to growers for improved WUE without sacrificing yield.

ACKNOWLEDGEMENTS

We thank the staffs at the National Peanut Research Laboratory and specifically Kathy Gray for providing the technical laboratory expertise to complete this study. We also wish to thank Larry Powell, William Pearce, Eva Whitehead, Latoya Rucker, and Chris Butts who provided invaluable field collection, sample processing, and general assistance and Graeme Wright and Rachaputi Nageswara Rao for technical advice and very useful discussions.

REFERENCES

Bell MJ, Wright GC (1994). The N_2-fixing capacity of peanut cultivars with differing assimilate partitioning characteristics. Aust. J. Agric. Res., 45: 1455-1468.

Brown RH, Byrd GT (1996). Transpiration efficiency, specific leaf weight, and mineral concentration in peanut and pearl millet. Crop Sci., 36: 475-480.

Craufurd PQ, Wheeler TR, Ellis RH, Summerfield RJ, Williams JH (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.

Delwiche CC, Zinke PJ, Johnson CM, Virginia A (1979). Nitrogen

isotope distribution as a presumptive indicator of nitrogen fixation. Bot. Gaz. 140 Suppl., 65-69.
Farquhar GD, Ehleringer JR, Hubick KT (1989). Carbon isotope discrimination and photosynthesis. Ann. Rev. Plant Physiol., 40: 503-537.
Farquhar GD, O'Leary MH, Berry JA (1982). On the relationship between carbon isotope discrimination and intercellular carbon dioxide concentration in leaves. Aust. J. Plant Physiol., 9:121-137.
Farquhar GD, Richards RA (1984). Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. Aust. J. Plant Physiol., 11: 539-552.
Guehl JM, Domenach AM, Bereau M, Barigah TS, Casabiance H, Ferhi A, Garbaye J (1998). Functional diversity in an Amazonian rainforest of French Guyana: a dual isotope approach ($\delta^{15}N$ and $\delta^{13}C$). Oecologia, 116: 316-330.
Handley LL, Odee D, Scrimgeour CM (1994). $\delta^{15}N$ and $\delta^{13}C$ patterns in savanna vegetation: dependence on water availability and disturbance. Functional Ecol., 8: 306-314.
Hatfield PM, Wright GC, Tapsall WR (1989). A large retractable, low cost and relocatable rainout shelter design. Exp. Agric., 26: 57-62.
Hubick KT (1990). Effects of nitrogen source and water limitation on growth, transpiration efficiency and carbon-isotope discrimination in peanut cultivars. Aust. J. Plant physiol., 17: 413-430.
Hubick KT, Farquhar GD, Shorter R (1986). Correlation between water-use efficiency and carbon isotope discrimination in diverse peanut (*Arachis*) germplasm. Aust. J. Plant Physiol., 13: 803-816.
Hubick KT, Shorter R, Farquhar GD (1988). Heritability and genotype x transpiration efficiency in peanut (*Arachis hypogaea* L.). Aust. J. Plant Physiol., 15: 799-813.
Knight JD, Verhees F, Van Kessel C, Slinkard AE (1993). Does carbon isotope discrimination correlate with biological nitrogen fixation? Plant and Soil, 153: 151-153.
Lal C, Hariprasanna K, Rathnakumar AL, Gor HK, Chikani BM (2006). Gene action for surrogate traits of water-use efficiency and harvest index in peanut (*Arachis hypogaea*). Ann. Appl. Biol., 148: 165-172.
Lamb MC, Davidson JI Jr, Childre JW, Martin NR Jr (1997). Comparison of peanut yield, quality, and net returns between nonirrigated and irrigated production. Peanut Science, 24: 97-101.
Monje OA, Bugbee B (1992). Inherent limitations of nondestructive chlorophyll meters: a comparison of two types of meters. Hortscience, 27: 69-71.
Nageswara Rao RC, Talwar HS, Wright GC (2001). Rapid assessment of specific leaf area and leaf nitrogen in peanut (*Arachis hypgaea* L.) using a chlorophyll meter. J. Agron. Crop Sci., 186: 175-182.
Nageswara Rao RC, Udaykumar M, Farquhar GD, Talwar HS, Prasad TG (1995). Variation in carbon isotope discrimination and its relationship to specific leaf area and ribulose-1, 5-bisphosphate carboxylase content in groundnut genotypes. Aust J. Plant Physiol., 22: 545-551.
Nageswara Rao RC, Williams JH, Wadia KDR, Hubick KT, Farquhar GD (1993). Crop growth, water-use efficiency and carbon isotope discrimination in groundnut (*Arachis hypogaea* L.) genotypes under end-of season drought conditions. Ann. appl. Biol., 122: 357-367.
Nageswara Rao RC, Wright GC (1994). Stability of the relationship between specific leaf area and carbon isotope discrimination across environments in peanut. Crop Sci., 34: 98-103.
SAS (1997). JMP Statistical Discovery Software. Cary, NC, USA, SAS Institute Inc.
Schulze ED, Gebauer G, Ziegler H, Lange OL (1991). Estimates of nitrogen fixation by trees on an aridity gradient in Namibia. Oecologia, 88: 451-455.
Sheshshayee MS, Bindumadhava H, Rachaputi NR, Prasad TG, Udayakumar M, Wright GC, Nigam SN (2006). Leaf chlorophyll concentration relates to transpiration efficiency in peanut. Ann. Appl. Biol., 148: 7-15.
Songsri P, Jogloy S, Holbrook CC, Kesmala T, Vorasoot N, Akkasaeng C, 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. Agric. Water Manag., 96: 790-798.
Songsri P, Jogloy S, Kesmala T, Vorasoot N, Akkasaeng C, Patanothai A, Holbrook CC (2008). Heritability of drought resistance traits and correlation of drought resistance and agronomic traits in peanut. Crop Sci., 48: 2245-2253.
Sprent JI, Geoghegan IE, Whitty PW, James EK (1996). Natural abundance of $\delta^{15}N$ and $\delta^{13}C$ in nodulated legumes and other plants in the cerrado and neighbouring regions of Brazil. Oecologia, 105: 440-446.
Stansell JR, Shepherd JL, Pallas JE, Bruce RR, Minton NA, Bell DK, Morgan LW (1976). Peanut responses to soil water variables in the southeast. Peanut Sci., 3: 44-48.
Udayakumar M, Sheshshayee MS, Nataraj KN, Bindu Madhava H, Devendra R, Aftab Hussain IS, Prasad TG (1998). Why has breeding for water use efficiency not been successful? An analysis and alternate approach to exploit this trait for crop improvement. Current Sci., 74: 994-1000.
Upadhyaya HD (2005). Variability for drought resistance related traits in the mini core collection of peanut. Crop Sci., 45: 1432-1440.
Virginia R, Delwiche CC (1982). Natural ^{15}N abundance of presumed N_2-fixing and non-fixing plants from selected ecosystems. Oecologia, 54: 317-325.
Wanek W, Arndt SK (2002). Difference in $\delta^{15}N$ signatures between nodulated roots and shoots of soybean is indicative of the contribution of symbiotic N_2 fixation to plant N. J. Exp. Bot., 53: 1109-1118.
Wright GC, Hubick KT, Farquhar GD (1988). Discrimination in carbon isotopes of leaves correlates with water-use efficiency of field-grown peanut cultivars. Aust. J. Plant Physiol., 15: 815-825.
Wright GC, Hubick KT, Farquhar GD, Nageswara Rao RC (1993). Genetic and environmental variation in transpiration efficiency and its correlation with carbon isotope discrimination and specific leaf area in peanut. *In* Ehleringer JR, Hall AE, Farquhar GD (eds.), Stable Isotopes and Plant Carbon-Water Relations. Academic Press, Inc., San Diego, CA, USA.
Wright GC, Nageswara Rao RC, Basu MS (1996). A physiological approach to the understanding of genotype by environment interactions – a case study on improvement of drought adaptation in groundnut. *In* Cooper M, Hammer GL (eds.), Plant Adaptation and Crop Improvement. CAB International, Wallingford, Oxfordshire, UK.
Yoo CY, Pence HE, Hasegawa PM, Mickelbart MV (2009). Regulation of transpiration to improve crop water use. Crit. Rev. in Plant Sci., 28: 410-431.