Plant Soil (2010) 330:139–148
DOI 10.1007/s11104-009-0185-9

REGULAR ARTICLE

Genotypic variability among peanut (*Arachis hypogea* L.) in sensitivity of nitrogen fixation to soil drying

M. Jyostna Devi · Thomas R. Sinclair · Vincent Vadez

Received: 20 July 2009 / Accepted: 25 September 2009 / Published online: 13 October 2009

Abstract Peanuts *(Arachis hypogea* L.) are often grown on sandy soils and drought stress can be a major limitation on yield. In particular, loss in nitrogen fixation activity associated with soil drying might be limiting due to the need for high nitrogen amounts in both vegetative tissues and seeds of peanut. This study examined the response of nitrogen fixation of intact plants of seventeen peanut genotypes when subjected to soil drying in pots over approximately a 2-wk period. A large range in the sensitivity of nitrogen fixation to soil drying was observed among the seventeen genotypes. Genotype ICGV86015, in particular, was found to have nitrogen fixation that was especially tolerant of soil drying. Significant positive ($P<0.0001$) correlation was found between the soil water content at which nitrogen fixation began decreasing and the amino acid concentration in the leaves of severely stressed plants.

Keywords Amide · *Arachis hypogea* L. · Drought · Nitrogen fixation · Peanut · Soil drying · Ureide

Responsible Editor: Euan K. James.

M. J. Devi · T. R. Sinclair (✉)
Agronomy Department, University of Florida,
P.O. Box 110965, Gainesville, FL 32611–0965, USA
e-mail: trsincl@ifas.ufl.edu

V. Vadez
ICRISAT (International Crops Research Institute
for the Semi-Arid Tropics), GT Biotechnology,
Patancheru 502324, Andhra Pradesh, India

Introduction

Drought affects nitrogen fixation and related traits in peanuts (*Arachis hypogea* L.) (Pimratch et al. 2008a, b) and is a major limiting factor of yield (Wright et al. 1991, 1994; Nautiyal et al. 1999). Nitrogen fixation activity of peanut has been studied under both well-watered (Sen and Weaver 1984) and water deficit conditions (Venkateswarlu et al. 1989, 1990; Sinclair et al. 1995; Pimratch et al. 2008a, b; Wunna et al. 2009). In comparative studies of peanuts with other legume species subjected to drought, nitrogen fixation of peanut was found to be relatively insensitive (DeVries et al. 1989a, b; Venkateswarlu et al. 1989; Sinclair and Serraj 1995).

Several parameters such as nodule weight and number, biomass production, shoot dry weight and nitrogenase activity have been used as indirect measurements of nitrogen fixation activity in peanut (Sinclair et al. 1995; Pimratch et al. 2008a, b; Wunna et al. 2009). Nitrogenase activity has been measured by incubating detached root systems in a polypropylene bottle filled with 1:9 acetylene:air for an hour (Venkateswarlu et al. 1989, 1990; Pimratch et al. 2008a). The disadvantage of this approach is that detaching roots interrupts plant metabolic and transport processes that are likely to influence nitrogenase activity in the intact plant. In addition, when attempting to study the effect of drought on N2 fixation, it is important to have an accurate record of the water-stress level. Sinclair et al. (1995) discussed the complications of the methodology and suggested an approach in which nitrogenase activity can be

expressed as a function of transpirable soil water. Unlike earlier studies where nitrogenase activity was measured only 35, 60 and 90 days after emergence at field capacity, 2/3 and 1/3 available soil water level (Pimratch et al. 2008a), the approach suggested by Sinclair et al. (1995) can be used to evaluate nitrogenase activity of each plant daily over the complete soil drying cycle from well-watered conditions to severe plant stress.

A precise understanding of the factors involved in the regulation and limitation of nitrogen-fixation activity may help to identify peanut genotypes that are useful for developing drought-tolerant cultivars. One possible mechanism for decreased nitrogen fixation in response to water deficit, is that nitrogen fixation may be very closely coupled to gas exchange capacity of leaves. That is, stomatal closure in response to soil drying could limit transpiration rate and CO2 assimilation such that nitrogen fixation activity might be simultaneously decreased. In soybean (*Glycine max* (L.) Merr.), however, nitrogen fixation activity is highly sensitive to soil drying, and in nearly all tested cultivars decreased well in advance of decreases in leaf gas exchange (Serraj and Sinclair 1997).

A second hypothesis to explain the decline of nitrogen fixation in response to soil drying is feedback inhibition induced by nitrogenous compounds in the plant (Parsons et al. 1993; Vadez et al. 2000; Parsons and Sunley 2001; Serraj et al. 2001; King and Purcell 2005; Marino et al. 2007). In soybean, ureides have been identified as having a major feedback role in nitrogen fixation inhibition (Serraj and Sinclair 1996; Vadez et al. 2000; Serraj et al. 2001; King and Purcell 2005). It has been suggested that specific amino acides, including glutamine (Neo and Layzell 1997), and asparagine (Bacanamwo and Harper 1997), might be more directly involved in the nitrogen feedback in soybean. Additionally, it has been proposed that both ureides and aspartate in nodules and free amino acids in the leaves are involved in the feedback inhibition of nitrogen fixation in soybean under water deficit (King and Purcell 2005).

In peanut, ureides do not appear to be a major transport product since amino acid concentrations are much higher in the xylem sap (Peoples et al. 1986, 1991). However, the link between amino acid concentration in plants and the sensitivity of nitrogen fixation to soil drying has not been examined across peanut genotypes. Therefore, the objectives of the study were to 1) observe the differences among peanut genotypes in sensitivity of nitrogen fixation to soil drying, 2) determine the metabolites of N2 fixation in both well-watered and water-deficit treatments, and 3) study the accumulation of metabolic products during drought and its relation to nitrogen fixation inhibition in peanut cultivars.

Materials and methods

Plant material

Seventeen peanut genotypes were selected to study nitrogen fixation response under drought stress. These genotypes were selected because they had exhibited a range of sensitivity to water-deficit conditions as gauged by transpiration efficiency (Devi et al. 2009). To test all genotypes, four sets of experiments were performed from January 2008 to July 2008. Each set included different types of genotypes with a maximum number of five genotypes (Table 1). Each experiment was undertaken in a greenhouse in Gainesville, FL (29° 38′N, 82°22′W). The temperature in the greenhouse for all experiments was regulated at 27°C day and 21°C night. Incandescent lamps were used to extend the daylength in all experiments to 15.5 to 16 h.

Plants were grown in pots (10-cm diameter and 30-cm tall) constructed from PVC pipe. An end cap was glued to the bottom of the pots through which a fitting for introducing gas into the pot was installed for measurements of acetylene reduction rate. The fitting was left open when acetylene reduction rate was not being measured so that it served as a drainage hole for the pot. A toilet flange was glued to the top of the pot to which a lid could be sealed when measuring acetylene reduction rate. Prior to measuring acetylene reduction rates, a two-piece lid was bolted on to the pot and sealed around the stem of the plant so that the entire pot was gas tight and an air:acetylene gas mixture could be flowed through the pots.

Pots were filled with top soil (Robin Hood Timber Co, Adel, GA) that was first sieved through a 0.5-cm sieve and then a 0.2-cm sieve to eliminate bark and large pieces of organic matter. Prior to sowing (dates for each experiment presented in Table 1), the seeds were treated with 2% ethrel to break the dormancy and the pots were inoculated with a mixture of rhizobia (Peanut Bacteria, Southern States Cooperative, Richmond, Virginia). Plants were maintained in a

Table 1 List of genotypes included in each experiment, date of sowing and dates of experiments conducted

Experiment	Genotype	Date of sowing	Dates of experiment
Experiment 1	ICG 3179 ICGV 86015	22 Nov 2007	4 Jan 2008 to 22 Jan 2008
	ICGV 86388		
	ICGV 91284		
	Kopergagon 3		
Experiment 2	ICGV 86564 ICGV 86699	12 Feb 2008	4 Apr 2008 to 19 Apr 2008
	PI 544346		
Experiment 3	ICG 11376 ICGV 87128	18 Feb 2008	29 Apr 2008 to 15 May 2008
	PI 259747		
	Gajah		
	TMV 2		
Experiment 4	ICGS 44 ICGV 86031	23 May 2008	10 Jul 2008 to 23 Jul 2008
	TAG 24		
	ICGV 87141		

well-watered condition until the start of each dry-down experiment. Plants in all experiments exhibited substantial nodulation and nitrogen fixation rates.

Peanut plants, in contrast to species with a long basal stem length, do not naturally have a stem length around which the pot lid could be sealed. To overcome this problem, the peanut plants were grown to allow access to the basal stem area that is normally below the soil surface. A 7 to 10-cm length of foam pipe insulation tube wrapped with aluminum foil was positioned vertically on the soil surface and filled with soil. A single seed was sown in the insulation tube in each pot and allowed to grow normally. When it was time to make ARA measurements, the foam insulation and soil was gently removed exposing the basal stem area of the plant. The two-piece lid could then be installed around this basal stem area to seal the pot. Nine to eleven replicates were sown for each genotype. All plants in each experiment were grown under well-watered conditions for minimum of 6 weeks until they reached a height of at least 20 to 25 cm.

Measurement of acetylene reduction activity

Once the plants had reached 20- to 25-cm height, a dry-down experiment was initiated (dates for each experiment are presented in Table 1). Measurements were made simultaneously on 40 pots in each experiment. Depending on whether four or five genotypes were studied in an experiment, eight or ten pots were selected for each genotype. Depending on the number of pots per genotype, three or four pots were maintained in a well-watered condition and five or six pots were subjected to the water-deficit treatment. The day before the start of the experiment, all pots were saturated with water and left overnight to drain excess water. On the following morning, all pots were sealed with the two-piece lid and weighed. Subsequently, each afternoon the pots were reweighed to determine transpiration rates as the difference between successive weights. Based on the first-day transpiration rates, plants were divided between the well-watered and drought-stressed treatments so that there was a uniform distribution of plants between the two treatments based on plant transpiration potential. Well-watered plants were watered daily to a weight 100 g less than the fully wet condition to avoid fully saturating the soil each day. Water-deficit plants were watered if necessary on each day when transpiration loss exceeded 70 g so that the net loss for that day was about 70 g. Consequently, soil drying was extended over two weeks.

Each afternoon during the experimental measurement period, acetylene reduction rate (ARA) was measured for each plant by flowing a 1:9 volume mixture of C2H2:air through all pots simultaneously at 1 L min^{-1}. The gas mixture was flowed for 15 min to allow equilibration of the gas mixture in the pot

and a stabilization of ethylene production measured in the exit from the pot. After the stabilization period, three gas samples were collected at the outlet port in the lid using 1-cm^3 syringes. Immediately following collection of the gas samples, acetylene flow was removed and only air was allowed to flow through the pots for at least an additional hour to fully remove acetylene from the pots. Gas samples were analyzed with a gas chromatograph (Model 5710A, Hewlett-Packard Corp., Palo Alto, CA) for ethylene concentration. The short exposure time of the plants to the acetylene and ethylene in this system has resulted in no evidence of ARA decreases as a result of repeated exposure of soybean (Sall and Sinclair 1991) or peanut (Sinclair et al. 1995) rhizosphere to acetylene.

Average ethylene concentration for each pot was calculated as the average of the three gas samples. A mean ARA on each day for well-watered plants was calculated using the results from the replicate well-watered plants. For each drought-stressed plant, a ratio was calculated each day between its ARA and the mean of the well-watered plants. To overcome plant-to-plant variability in the ARA values among drought-stressed plants, the ARA ratio for these plants was further normalized so that ARA for each drought-stressed plant had an average value of 1.0 during initial days of the experiment before soil drying inhibited ARA. In a similar way, transpiration rate ratio and normalized transpiration ratio were calculated for each drought-stressed plant. The experiment was terminated for each drought-stressed plant when daily normalized transpiration ratio was below 0.1, i.e. when the transpiration of drought-stressed plants was less than 10% of the well-watered plants. Fraction of transpirable soil water (FTSW) of each day was calculated as the amount of transpirable water remaining in the soil divided by the total transpirable soil water (initial weight minus final weight). No difference in total transpirable soil water was observed among the genotypes.

Ureide and amino acid measurements

Ureide and amino acid concentrations were measured in the leaf tissue collected at the end of the experiments. The leaves of both well-watered and drought-stressed plants were dried and 50 mg of ground tissue was used for the extraction of ureides and amino acids. The extraction procedure was similar for both. Ground leaf tissue was boiled in 1 ml of 0.2 M NaOH for 30 min. The samples were brought to room temperature and then centrifuged for 10 min at 10,000 RPM. The samples were then stored in refrigerator.

Ureides concentration was measured for a 300 μl aliquot of supernatant using the colorimetric method described by Trijbels and Vogel (1966). A 100 μl aliquot of supernatant was used to measure amino acid concentration using a modification of the ninhydrin method (Yemm and Cocking 1955). For the amino acid analysis, asparagine was used as a standard.

Statistical analysis

Acetylene reduction activity was examined as a function of FTSW. Individual daily values of normalized ARA for all drought-stressed replicates of a genotype were included in the analysis. A two-segment linear regression analysis was used to determine the FTSW value, i.e. threshold (X_0), at which ARA began to decrease (GraphPad Prism 2.0 Software Inc., San Diego, CA, 1996). The regression model for the analysis was two intersecting linear regressions,

$$Y1 = \text{slope1}*\text{FTSW} + \text{intercept1 at FTSW} < X_0$$

$$Y2 = Y \text{ at } X_0 + \text{slope2}*(\text{FTSW} - X_0) \text{ at FTSW} > X_0$$

In these analyses, the value of Y2 was defined equal to 1.0 as a result of the normalization of the data when the soil was still wet. The threshold FTSW where the decline in ARA was initiated (X_0) was calculated from regression analysis using GraphPad Prism software to obtain the best overall fit for the two-segment model.

The mean values of the threshold FTSW, ureide and amino acid concentrations among the four experiments were compared using an ANOVA (completely randomized design). Finding no significant effect of the experiment, the data were combined and all genotypes compared by the Tukeys method.

Results

FTSW threshold for ARA

The two-segment model for the response of ARA to soil drying generally fit the data well for each

genotype (Fig. 1). There was little change in the normalized ARA at high soil water content, then a linear decrease in normalized ARA to a small positive value at FTSW equal to zero. The correlation coefficient for the two-segment regression ranged from 0.61 for ICGV 86015 to 0.95 for ICGV 86564 (Table 2). The ability to describe these ARA results with the two-segment model results compare favorably with previous results for peanut (Sinclair et al. 1995) and soybean (Vadez et al. 2000).

There was no evidence that there was any bias in the results by obtaining results in four experiments. Not only was there no difference in the amount of water extracted from the pots among the four experiments, but there was no significant difference in the breakpoint in transpiration rate among the four experiments (Devi et al. 2009). Similarly, there was not significant difference in the breakpoint for ARA among the four experiments as shown in the ANOVA analysis.

A significant difference among individual peanut genotypes in their FTSW threshold for declining ARA with drying soil was observed (Table 2). The range for the threshold was from 0.28 for the most tolerant genotype ICGV 86015 to 0.59 for the most sensitive genotype ICGV 86564. The threshold of ICGV 86015 was statistically lower than only three of the most sensitive lines, however. The sensitive line ICGV 86564 was more sensitive than the seven most tolerant lines. Due to plant-to-plant variation in the ARA measurements, statistical difference in the threshold was not shown among 14 lines even though their threshold range was from 0.31 to 0.50.

Substantial variation in the FTSW threshold for transpiration was previously reported among these genotypes (Devi et al. 2009). For comparison of the sensitivity to declining soil water content, the FTSW threshold for decline in ARA was plotted against the FTSW threshold for decline in transpiration (Fig. 2). For nearly all genotypes, the ARA threshold was less than the threshold for transpiration confirming comparative insensitivity of nitrogen fixation in peanut to soil drying. While the correlation was not high (r^2=0.25)

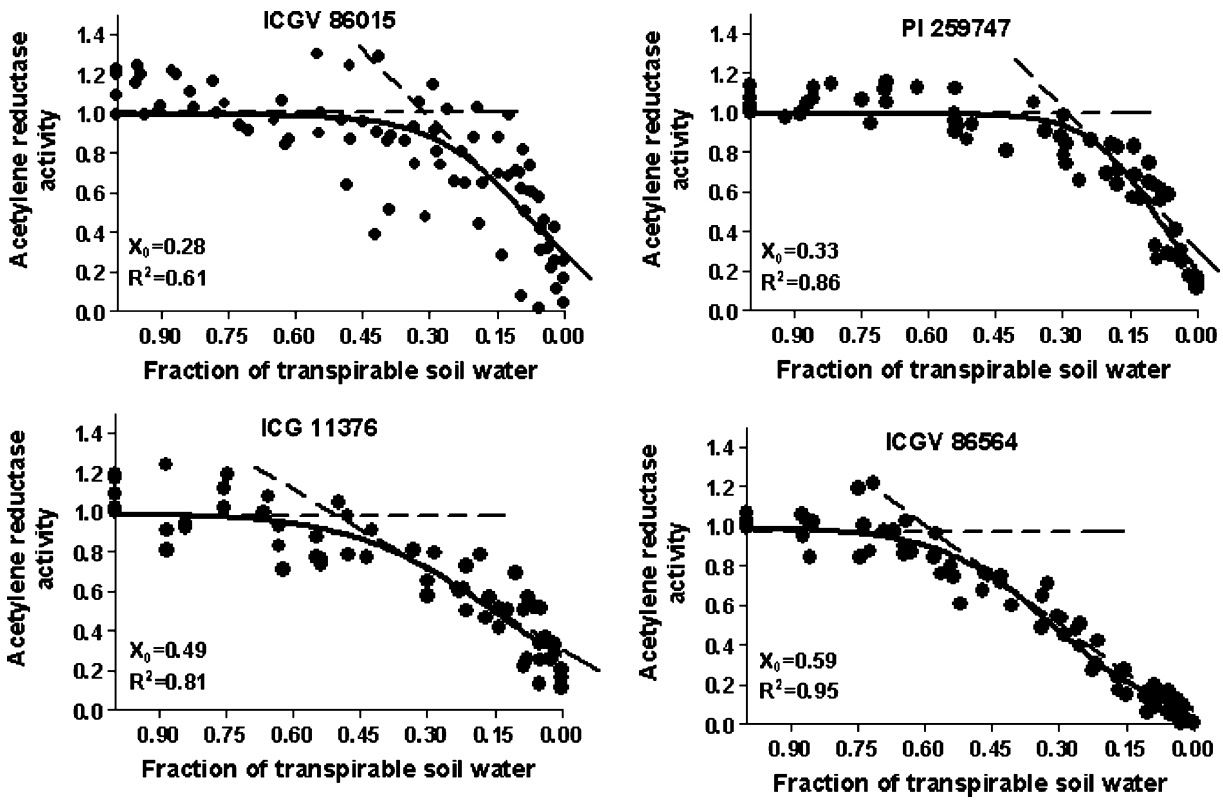

Fig. 1 Acetylene reduction activity of four representative peanut genotypes plotted against FTSW. *Solid lines* in each figure are the regression results using two-segment linear analysis

Table 2 FTSW-threshold values acetylene reduction activity in seventeen genotypes with their R^2, Standard error (±) and confidence limit. The FTSW—threshold values with the similar letter had no significant variation based on their confidence limits

Genotype	FTSW-threshold for ARA	R^2	S.E. (±)	Confidence limit
ICGV 86015	0.28 a	0.61	0.03	0.23 to 0.33
ICGV 86388	0.31 ab	0.58	0.04	0.26 to 0.43
PI 259747	0.33 ab	0.86	0.02	0.29 to 0.37
TMV 2	0.35 abc	0.88	0.04	0.29 to 0.46
ICGV 91284	0.37 abc	0.81	0.04	0.29 to 0.40
ICGV 86699	0.37 abc	0.88	0.04	0.29 to 0.46
Gajah	0.38 abc	0.89	0.05	0.37 to 0.50
Kopergagon 3	0.40 abcd	0.67	0.07	0.36 to 0.61
ICGV 87141	0.41 abcd	0.65	0.05	0.35 to 0.52
ICGV 86031	0.44 abcd	0.78	0.07	0.34 to 0.52
ICGS 44	0.45 abcd	0.68	0.03	0.40 to 0.58
PI 544346	0.45 abcd	0.82	0.03	0.38 to 0.49
TAG 24	0.45 abcd	0.91	0.03	0.39 to 0.49
ICG 3179	0.47 abcd	0.70	0.11	0.35 to 0.60
ICG 11376	0.50 bcd	0.81	0.06	0.40 to 0.58
ICGV 87128	0.55 cd	0.82	0.03	0.51 to 0.66
ICGV 86564	0.59 d	0.95	0.03	0.56 to 0.64

between the two variables, there was a significant trend (P=0.03) and a low threshold for transpiration was associated with a low threshold for ARA.

Leaf ureide concentration

Concentration of leaf ureide in both well-watered and drought-stressed plants was very low. In both treatments, leaf ureide concentrations were less than 3 μmol g^{-1} (Fig. 3). Leaf ureide concentrations were found to range from 1.45 to 2.38 μmol g^{-1} in the well-water regime and 1.04 to 2.89 μmol g^{-1} in the drought-stress treatment, with a mean across genotypes under well-water regime of 1.83 μmol g^{-1} and a mean under drought stress of 1.74 μmol g^{-1}. There was no consistent trend within genotypes in whether the well-watered or drought-stressed plants had the higher ureide concentration. Since leaf ureide concentrations were low, it is not a surprise that there was no correlation between the ARA threshold across genotypes and the leaf ureide concentration in either well-watered leaves or drought-stress leaves (data not shown).

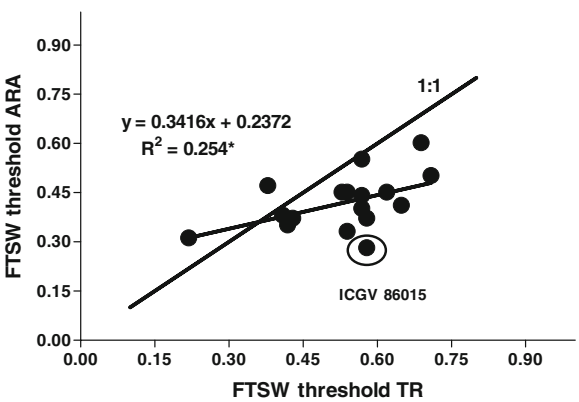

Fig. 2 FTSW threshold for decline in ARA is plotted against FTSW threshold for decrease in transpiration rate (*TR*) of seventeen peanut genotypes (P=0.03)

Leaf amino acid concentration

Amino acid concentrations in the leaves of all peanut genotypes (Fig. 4) were high relative to the ureide concentrations both under well-watered and drought-stressed treatments. The concentrations of the amino acids were more than 100-fold of the ureide concentrations. Significant genotypic differences in leaf amino acid concentrations in both the well-watered and drought-stressed treatments were identified (Fig. 4). Under well-watered conditions, leaf amino acid concentration ranged from 305 μmol g^{-1} for ICGV 86388 to 467 μmol g^{-1} for ICGS 44.

Leaf amino acid concentration was equal to or greater in drought-stressed leaves as compared to well-watered leaves depending on genotypes. The lowest leaf amino acid concentration for the drought-

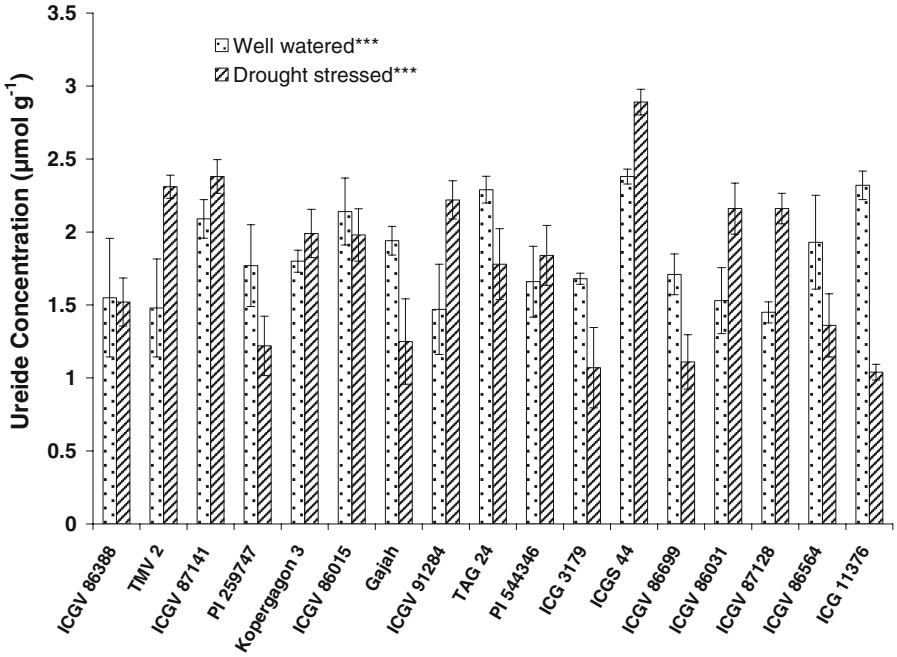

Fig. 3 Average concentration of ureides in the seventeen peanut genotypes both under well-watered and drought-stressed treatments. Standard error values are indicated for each average concentration. ANOVA analysis indicated significant differences ($P<0.001$) among genotypes within each watering treatment

stressed leaves was again observed in ICGV 86388 with a value of 358 µmol g^{-1}. Several genotypes including ICG 11376, ICGV 863464, and ICGV 87128 had leaf amino acid concentrations greater than 600 µmol g^{-1}. The ARA threshold for decrease with soil drying gave an interesting comparison with leaf amino acid concentration. There was no correlation between the ARA threshold and the amino acid

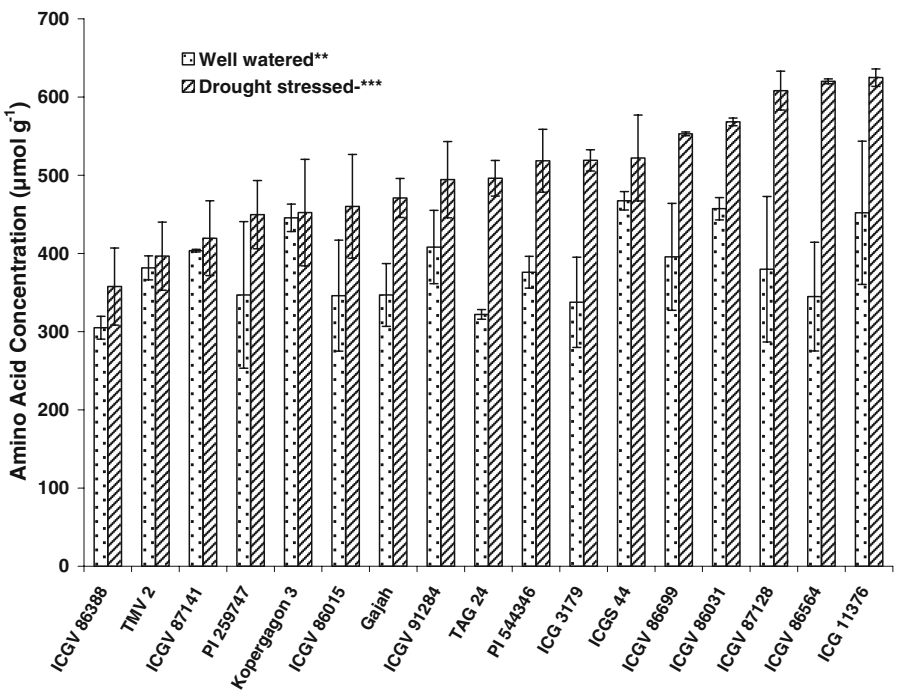

Fig. 4 Mean amino acid concentration and standard error of seventeen peanut genotypes in well-water and water-deficit treatments. Based on ANOVA analysis, genotypic differences were significant in both the well-watered treatment ($P<0.01$) and water-deficit treatment ($P<0.001$)

concentration of leaves collected from well-watered plants (Fig. 5). By contrast, the ARA threshold was significantly correlated ($P<0.0001$) with leaf amino acid concentration accumulated under dry conditions (Fig. 5). That is, higher amino acid concentrations under drought-stress conditions were positively correlated with observations for an increased ARA threshold.

Discussion

This study identified large differences among 17 genotypes in their nitrogen fixation sensitivity to soil drought stress based on data obtained throughout a dry-down cycle. Pimratch et al. (2008a, b) also observed genetic variation but their conclusions were based on limited observations at field capacity and 2/3 available soil water evaluated 60 and 90 days after emergence. The conclusion from our study and the study of Pimratch et al. (2008a, b) give strong support to the proposal that genetic variation exists in nitrogen fixation tolerance to soil drying within the peanut germplasm. Those lines with lower thresholds for decline in ARA are candidates for possible breeding efforts to develop improved cultivars for water-deficit conditions as has been done in soybean (Sinclair et al. 2007).

The conclusion of large genetic variation in nitrogen fixation tolerance is somewhat inconsistent with the general impression that nitrogen fixation in peanut is drought insensitive. Sinclair and Serraj (1995) previously concluded that nitrogen fixation of peanut is tolerant to soil drying as compared to other grain legumes. Using the same experimental set up as used in the current study, Sinclair et al. (1995) concluded that there was substantial tolerance of nitrogen fixation to soil drying for the six commercial cultivars they studied. Other studies also with a limited number of cultivars concluded that nitrogen fixation of peanut was relatively insensitive to soil drying (DeVries et al. 1989a, b; Venkateswarlu et al. 1989). The current results indicate that the conclusion about insensitivity of nitrogen fixation in peanut to soil drying may be a result of studying only a limited number of genotypes selected for commercial use. This conclusion based on previous studies may indicate that empirical plant breeding has been successful in incorporating nitrogen fixation drought tolerance in commercial cultivars even though a range of sensitivity exists within the germplasm. Success in breeding for high-yielding peanut on sandy soils likely would have favored negative selection of those lines with high sensitivity of nitrogen fixation to drying soil.

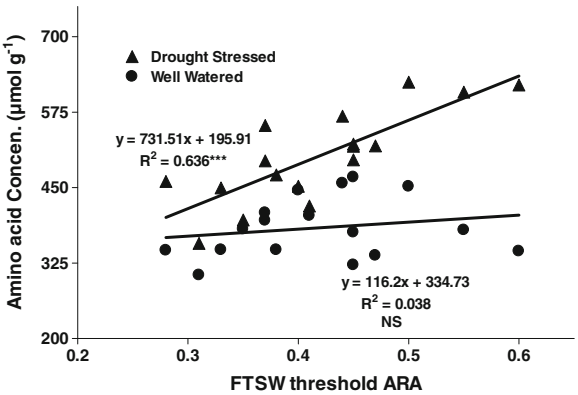

Fig. 5 Regression analysis of FTSW-threshold for ARA and concentration of amino acids measured in the leaves collected at the end of the experiment from both well-watered and drought-stressed treatments. The relationship was non-significant under well-watered treatment and significant ($P<0.0001$) under water deficit

Comparison of the thresholds for ARA and transpiration showed a positive correlation between the two variables although the correlations coefficient was small ($r2=0.25$). However, these data showed for most genotypes that ARA was less sensitive to soil drying that transpiration. The response of ICGV 86015 is especially intriguing because it had the lowest ARA threshold, yet had a comparatively high threshold for transpiration (Fig. 2). The combination of these responses might be particularly well suited for water-deficit conditions. This genotype is prone to soil water conservation by an early decline in transpiration with soil drying and the nitrogen fixation activity remains high until the soil dries to a very low level.

The leaf ureide and amino acid concentrations offer additional support obtained from a number of genotypes that the transport of nitrogen products from the nodules of peanut are amides rather than ureides as occurs in soybean and cowpea (Sinclair and Serraj 1995). The ureide concentrations in the leaves were only about one-hundredth of the amino acid concentrations across all genotypes. Peoples et al. (1986, 1991) previously reported very low ureide concentration in the sap of peanut and concluded that the main products of nitrogen fixation in peanut are amino acids.

Due to the low concentrations of ureides, it is not surprising there was no association between leaf ureide concentration and the threshold for decline in ARA. On the other hand, there was a positive association between leaf amino acid concentration under water-deficit conditions and the ARA threshold (Fig. 5). That is, those genotypes with greater ARA sensitivity to soil drying had accumulated greater concentrations of amino acid in water-deficit stress leaves. Amino acid concentrations measured when plants were under well-watered conditions, however, were not a predictor of ARA sensitivity to soil drying. While these results do not necessary confirm that a feedback response based on amino acid concentration is influencing nitrogen fixation activity, this is a hypothesis that certainly cannot be rejected based on these results.

The correlation among genotypes of leaf amino acid concentration of water-deficit stressed plants and ARA sensitivity indicates the possibility of a screen for examining a large number of genotypes. That is, a possible screen would involve subjecting a large number of genotypes to water-deficit stress and then harvesting leaves to measure amino acid concentrations. Those genotypes with low amino acid concentration would be candidates for greater nitrogen fixation tolerance to soil drying. Considering the scatter in these results, however, such a breeding screen might be viewed mainly as a negative screen to eliminate those genotypes with high amino acid concentrations as poor candidates for nitrogen fixation drought tolerance. Detailed direct tests of nitrogen fixation would probably still be needed to confirm nitrogen fixation tolerance in the positive candidates identified in the field screen.

Acknowledgments The senior author was supported by a USAID-linkage grant between ICRISAT and the University of Florida. The lines used in the study were previously identified from a project funded by the Generation Challenge Program (#2005-31 "Unlocking the genetic diversity in peanut's wild relatives with genomic and genetic tools").

References

Bacanamwo M, Harper JE (1997) The feedback mechanism of nitrate inhibition of nitrogenase activity in soybean may involve asparagine and/or products of its metabolism. Physiol Plant 100:371–377

Devi MJ, Sinclair TR, Vadez V, Krishnamurthy L (2009) Peanut genotypic variation in transpiration efficiency and stomatal closure during progressive soil drying. Field Crop Res (In press)

DeVries JD, Bennett JM, Albrecht SL, Boote KJ (1989a) Water relations, nitrogenase activity and root development of three grain legumes in response to soil water deficits. Field Crop Res 21:215–226

DeVries JD, Bennett JM, Boote KJ, Albrecht SL, Maliro CE (1989b) Nitrogen accumulation and partitioning by three grain legumes in response to soil water deficits. Field Crop Res 22:33–44

King CA, Purcell LC (2005) Inhibition of N_2 fixation in soybean is associated with elevated ureides and amino acids. Plant Physiol 137:1389–1396

Marino D, Frendo P, Ladrera R, Zabalza A, Puppo A, Arrese-Igor C, González EM (2007) Nitrogen fixation control under drought stress: localized or systemic? Plant Physiol 143:1968–1974

Nautiyal PC, Ravindra V, Zala PV, Joschi YC (1999) Enhancement of yield in groundnut following the imposition of transient soil-moisture-deficit stress during the vegetative phase. Exp Agric 35:371–385

Neo HH, Layzell DB (1997) Phloem glutamine and the regulation of O_2 diffusion in legume nodules. Plant Physiol 113:259–267

Parsons R, Sunley RJ (2001) Nitrogen nutrition and the role of root-shoot nitrogen signalling particularly in symbiotic systems. J Exp Bot 52:435–443

Parsons R, Stanforth A, Raven JA, Sprent JI (1993) Nodule growth and activity may be regulated by a feedback mechanism involving phloem nitrogen. Plant Cell Environ 16:125–136

Peoples MB, Pate JS, Atkins CA, Bergersen FJ (1986) Nitrogen nutrition and xylem sap composition of peanut (*Arachis hypogaea* L. cv. Virginia Bunch). Plant Physiol 82:946–951

Peoples MB, Atkins CA, Pate JS, Chong K, Faizah AW, Suratmini P, Nurhayati DP, Bagnall DJ, Bergersen FJ (1991) Re-evaluation of the role of ureides in the xylem transport of nitrogen in *Arachis* species. Physiol Plantarum 83:560–567

Pimratch S, Jogloy S, Vorasoot N, Toomsan B, Kesmala T, Patanothai A, Holbrook CC (2008a) Effect of drought stress on traits related to N_2 fixation in eleven peanut (*Arachis hypogaea L.*) genotypes differing in degrees of resistance to drought. Asian J Plant Sci 7:334–342

Pimratch S, Jogloy S, Vorasoot N, Toomsan B, Patanothai A, Holbrook CC (2008b) Relationship between biomass production and nitrogen fixation under drought stress conditions in peanut genotypes with different levels of drought resistance. J Agron Crop Sci 194:15–25

Sall K, Sinclair TR (1991) Soybean genotypic differences in sensitivity of symbiotic nitrogen fixation to soil dehydration. Plant Soil 133:31–37

Sen D, Weaver RW (1984) A basis for different rates of nitrogen fixation by the same strains of Rhizobium in peanut and cowpea root nodules. Plant Sci Lett 34:239–246

Serraj R, Sinclair TR (1996) Processes contributing to N2-fixation insensitivity to drought in the soybean cultivar Jackson. Crop Sci 36:961–968

Serraj R, Sinclair TR (1997) Variation among soybean cultivars in dinitrogen fixation response to drought. Agron J 89:963–969

Serraj R, Vadez V, Sinclair TR (2001) Feedback regulation of symbiotic N2 fixation under drought stress. Agronomie 21:621–626

Sinclair TR, Serraj R (1995) Legume nitrogen fixation and drought. Nature 378:344

Sinclair TR, Leilah AA, Schreffler AK (1995) Peanut nitrogen fixation (C_2H_2 reduction) response to soil dehydration. Peanut Sci 22:162–166

Sinclair TR, Purcell LC, King CA, Sneller CH, Chen P, Vadez V (2007) Drought tolerance and yield increase of soybean resulting from improved symbiotic N_2 fixation. Field Crop Res 101:68–71

Trijbels F, Vogel GD (1966) Degradation of allantoin by *Pseudomonas acidovirans*. Biochim Biophys Acta 113:292–301

Vadez V, Sinclair TR, Serraj R, Purcell LC (2000) Manganese application alleviates the water deficit-induced decline of N_2 fixation. Plant Cell Environ 23:497–505

Venkateswarlu B, Maheswari M, Karan NS (1989) Effects of water deficits on N_2 (C_2H_2) fixation in cowpea and groundnut. Plant Soil 114:69–74

Venkateswarlu B, Saharan N, Maheswari M (1990) Nodulation and N_2 fixation in cowpea and groundnut during water stress. Field Crop Res 25:223–232

Wright GC, Hubick KT, Farquhar GD (1991) Physiological analysis of peanut cultivar response to timing and duration of drought stress. Aust J Agric Res 42:453–470

Wright GC, Rao RCN, Farquhar GD (1994) Water-use efficiency and carbon-isotope discrimination in peanut under water-deficit conditions. Crop Sci 34:92–97

Wunna H, Jogloy S, Toomsan B, Sanitchon J (2009) Response to early drought for traits related to nitrogen fixation and their correlation to yield and drought tolerance traits in peanut (*Arachis hypogaea* L.). Asian J Plant Sci 8:138–145

Yemm EW, Cocking EC (1955) The determination of amino acids with ninhydrin. Analyst 80:209–213