Drought stress is one of the major environmental factors affecting peanut productivity and its effect can be economically devastating when occurring at critical growth stages. The objective of this study was to evaluate the effects of drought stress on symbiotic nitrogen fixation in various peanut genotypes. We repeated last year’s drought-stress experiment using the rainout shelter facilities at the USDA National Peanut Laboratory in Dawson, GA. Three treatments (irrigated control, middle-season and late-season drought) were applied to three separate rainout shelters. Two parental cultivars (Tifrunner and C76-16) and 14 recombinant lines (seven drought susceptible and seven drought tolerant genotypes) were planted in rainout shelters using a randomized complete block design within each drought treatment. The $^{15}$N natural abundance technique was used to evaluate differences in symbiotic nitrogen fixation among different genotypes under drought stress. The $^{13}$C natural abundance technique was used to determine carbon isotope discrimination, which has been shown to correlate with plant water use efficiency. Similar to last year, both middle- and late-season drought treatments negatively affected symbiotic nitrogen fixation; the middle-season drought treatment showed a greater reduction in the amount of N fixed than the late-season drought treatment. However, the extent of negative impact was less severe in 2016. Proportions of shoot N derived from the atmosphere varied among different genotypes. Under the middle-season drought, shoot N derived from N$_2$ fixation and carbon isotope discrimination were higher in the drought-tolerant genotypes than those in the susceptible ones. The most drought-tolerant genotype identified in our previous yield study had the highest N-fixing capacity under both drought treatments. There was a positive correlation between shoot N derived from N$_2$ fixation and carbon isotope discrimination under both middle- and late-season drought treatments. Experiments conducted in Year 3 confirmed the findings of Year 2. Overall, these results suggest that drought stress had a negative effect on symbiotic nitrogen fixation in peanut and the effect was more severe for mid-season drought.
Introduction

Peanut plants form symbiotic relationships with rhizobia, resulting in the fixation of atmospheric nitrogen, thus reducing or eliminating the need for nitrogen fertilization. Symbiotic nitrogen fixation is affected by the rhizobial strain involved, the genotype of the host plant, and environmental conditions. Different nitrogen fixation capabilities have been observed in different peanut cultivars. Symbiotic nitrogen fixation is known to be sensitive to soil drying, which tends to occur in sandy soils where peanut is commonly grown. Maximizing symbiotic nitrogen fixation during the development of high-yielding, drought tolerant peanut cultivars is critical for obtaining high yields without the application of expensive nitrogen fertilizers in peanut production. In this three-year project, we determined the effects of drought stress on symbiotic N$_2$ fixation in various peanut cultivars.

Materials and Methods

In Year 3, we repeated last year’s experiment to evaluate the effects of middle- and late-season drought on symbiotic N$_2$ fixation using the rainout shelter facilities at the USDA National Peanut Laboratory in Dawson, GA. Three treatments (irrigated control, middle-season and late-season drought) were applied to three separate rainout shelters. Two parental cultivars (Tifrunner and C76-16), seven drought susceptible and seven drought tolerant peanut genotypes were planted in the rainout shelters using a split-plot design with a randomized complete block design within. Middle- and late-season drought stress treatments were initiated 61 and 91 days after planting (DAP), respectively. Each drought treatment lasted for four weeks followed by a two-week recovery period. Peanut plants were harvested at the end of each drought treatment and two weeks after each drought treatment ended. Shoot samples were washed, oven-dried and weighed to obtain biomass data. Shoot samples were then ground up and analyzed for $^{15}$N and $^{13}$C natural abundance. The proportion of shoot N derived from N$_2$ fixation (%Ndfa) was calculated using the $^{15}$N data and carbon isotope discrimination calculated using the $^{13}$C data.

Results

Drought stress negatively affected symbiotic N$_2$ fixation in peanut for both middle- and late-season drought treatments; the impact of middle-season drought was greater than that of late-season (Figure 1). The proportions of shoot N derived from N$_2$ fixation for individual genotypes also differed significantly (Figure 2). The drought tolerant genotypes performed better in terms of N$_2$ fixation for the middle-season drought than the late-season drought. The most drought tolerant line (genotype 587) identified in our previous yield trials had the highest N-fixing capacity under both drought treatments. In addition, middle-season drought negatively affected carbon isotope discrimination in both years although no difference in carbon isotope discrimination was observed under late-season drought (Figure 3). Variabilities in carbon isotope discrimination were also observed among different genotypes. Under middle-season drought, carbon isotope discrimination values were higher in drought tolerant genotypes than drought susceptible ones. There was a positive correlation between %Ndfa and carbon isotope discrimination under both middle- and late-season drought treatments.

For the middle season drought treatment, N$_2$ fixation recovered in eight of the 16 genotypes studied after irrigation resumed; half of the eight were drought tolerant genotypes (Figure 4a). For the late season drought treatment, N$_2$ fixation in six genotypes showed recovery effect, half of which were drought tolerant genotypes (Figure 4b).
The differences in N₂ fixation between middle- and late-season drought treatments may be explained by the differences in environmental conditions (Figure 5). During the late-season drought treatment, the soil temperatures were lower and soil moisture levels were higher compared with the middle-season drought treatment.

Experiments conducted in Year 3 confirmed the findings of Year 2. Taken together, our results suggest that drought stress had a negative effect on symbiotic nitrogen fixation in peanut and the effect was more severe for mid-season drought.
Figure 1. Effect of middle (a) and late season (b) drought on symbiotic nitrogen fixation among individual peanut genotypes in 2016.
Figure 2. Genotype variability for symbiotic nitrogen fixation under middle-season (a) drought in late-season (b) drought in 2016. The filled columns indicate drought tolerant genotypes.
Figure 3. Carbon isotope discrimination among peanut genotypes: a) irrigation and mid-season drought in 2015; b) irrigation and mid-season drought in 2016; c) irrigation and late-season drought in 2015; and d) irrigation and late-season drought in 2016. Asterisks denote significant differences between irrigation and mid-season drought for each genotype (*P < 0.05; ** P < 0.01; *** P < 0.001; α = 0.05).
Figure 4. Recovery of symbiotic nitrogen fixation from drought treatments: a) middle season and b) late season.
Figure 5. Precipitation and soil moisture (at 20 cm) and temperature (at 20 cm) during the study period in 2016.