

## ORIGINAL RESEARCH ARTICLE

## Crop Physiology &amp; Metabolism

# Tolerance to mid-season drought in peanut can be achieved by high water use efficiency or high efficient use of water

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## Abstract

Peanut (*Arachis Hypogaea* L.) is an economic cash crop mainly planted in arid and semi-arid regions where drought causes approximately 20% yield losses every year. It has been demonstrated that drought tolerance can be achieved by two different mechanisms in plants: either by saving water or by extracting more water from the soil (water spender genotypes). The objective of this research was to screen for these two mechanisms of drought tolerance in peanut. Plants were grown in rainout shelters under irrigated conditions until mid-pod filling, then drought was induced using rainout shelters. Gas exchange parameters were measured regularly during the drought period and recovery. Genotypes PI 502120 and AU-NPL 17 were classified as water spender genotypes as they showed high yield,  $\Delta^{13}\text{C}$ , photosynthesis, and stomatal conductance under drought. This is the first time that water spender genotypes have been identified in peanut. On the other hand, genotypes Line-8 and AU16-28 were classified as water savers as they showed equally high yields but with low  $\Delta^{13}\text{C}$  and stomatal conductance and moderate photosynthesis under drought stress. In this study, water spender genotypes did not show a yield advantage in comparison with water savers.

## 1 | INTRODUCTION

Peanut (*Arachis Hypogaea* L.) is an important oilseed and human staple food crop as peanut seed contains 38–56% oil, 28–33% protein, 20% carbohydrates, and enriched vitamins and minerals (Food Data Central, 2019). In the Southeastern United States, peanut is used as a summer rotation crop with cotton to prevent boll weevil development in cotton (Johnson et al., 2001). In 2020, 3 million metric tonnes were produced in the United States on 526,000 ha (USDA, 2020), most of which are planted in Georgia, Alabama, Florida, North

Carolina, and Texas. Although four market types of peanuts are planted in the United States (i.e., Runner, Virginia, Valencia, and Spanish), the Runner type represents 80% of the production in the Southeastern United States and therefore is the focus of this study. The U.S. peanut belt is often subjected to periodic droughts and is often accompanied by high temperatures, which impact development and yield (Hamidou et al., 2013; Mittler, 2006).

Water availability is the single greatest abiotic constraint on crop plant productivity (Araus et al., 2002; Boyer, 1982), reducing yield in both irrigated and dryland cropping systems. In the United States, peanut is often grown in sandy soils with low water-holding capacity and in environments with variable rainfall. Therefore, without irrigation, peanut may be frequently subjected to drought stress. Drought results

**Abbreviations:** DAD, days after drought; EUW, efficient use of water;  $g_s$ , stomatal conductance; HI, harvest index; WUE, water use efficiency;  $\Delta^{13}\text{C}$ , carbon isotope discrimination (‰).

in the reduction of transpiration, and thus photosynthesis, which results in a reduction of biomass accumulation and yield (Sinclair et al., 2010; Tardieu & Tuberosa, 2010). For example, production losses in agriculture during the drought of 2012 were estimated to be \$30 billion in the United States (Ripley, 2015). It is estimated that the U.S. peanut industry loses \$50 million per year due to drought stress (USDA-ARS, 2019). Much of this loss is due to extended drought in nonirrigated (rainfed) fields, which is common in peanut production. For instance, in Alabama, only 10% of farmers have access to irrigation. In the southeastern United States, peanuts are mainly affected by mid-season drought (July–August), which significantly affects yield due to a decline in pegging and pod filling. To reduce the impacts of drought, it is crucial to conduct research and breed for drought-tolerant genotypes to improve peanut yield under dry conditions. This requires a better understanding of the physiological characteristics that make peanut tolerant to drought stress (Pimratch et al., 2007; Sinclair, 2011; Tardieu & Tuberosa, 2010; Tardieu et al., 2018).

In peanuts, different mechanisms associated with drought tolerance have been described including high water use efficiency (WUE; Vadez & Ratnakumar, 2016), quick transpiration reduction (Devi et al., 2010; Sinclair, 2011; Sinclair et al., 2018), deeper and more dense roots (Junjittakarn et al., 2014; Thangthong et al., 2017), and higher harvest index (HI; Vadez & Ratnakumar, 2016). Water use efficiency has been the focus of most studies. In very dry seasonal environments such as India or parts of Sub-Saharan Africa, genotypes having a high WUE or rapidly closing stomata can be more advantageous because these genotypes conserve soil moisture, increase the survival of the plant and maintain yield (Tardieu et al., 2018). The genotypes that utilize this mechanism of drought tolerance are called “water savers” or “isohydric” and are characterized by a rapid reduction in transpiration due to highly controlled stomatal opening under drought conditions (Blum, 2009; Tardieu et al., 2018). However, in locations where drought may be milder with periods of several weeks of drought interrupted by sporadic rains such as in the southeastern United States, these water saver genotypes may result in decreased yield due to stomatal closure and its effect on photosynthetic reduction, which limits growth and yield (Tardieu et al., 2018). In these environments, genotypes that have less control of the stomata opening and continue transpiring as the soil dries or that are able to obtain more water through a deeper root system may present a yield advantage as long as soil moisture is replenished before reaching the permanent wilting point (Blum, 2009; Tardieu et al., 2018). These genotypes are called “water spenders” or “Anisohydric” and are characterized as having a higher transpiration rate, efficient use of water (EUW), and photosynthesis rate under drought, leading to higher growth and yield under drought (Blum, 2009; Tardieu et al., 2018).

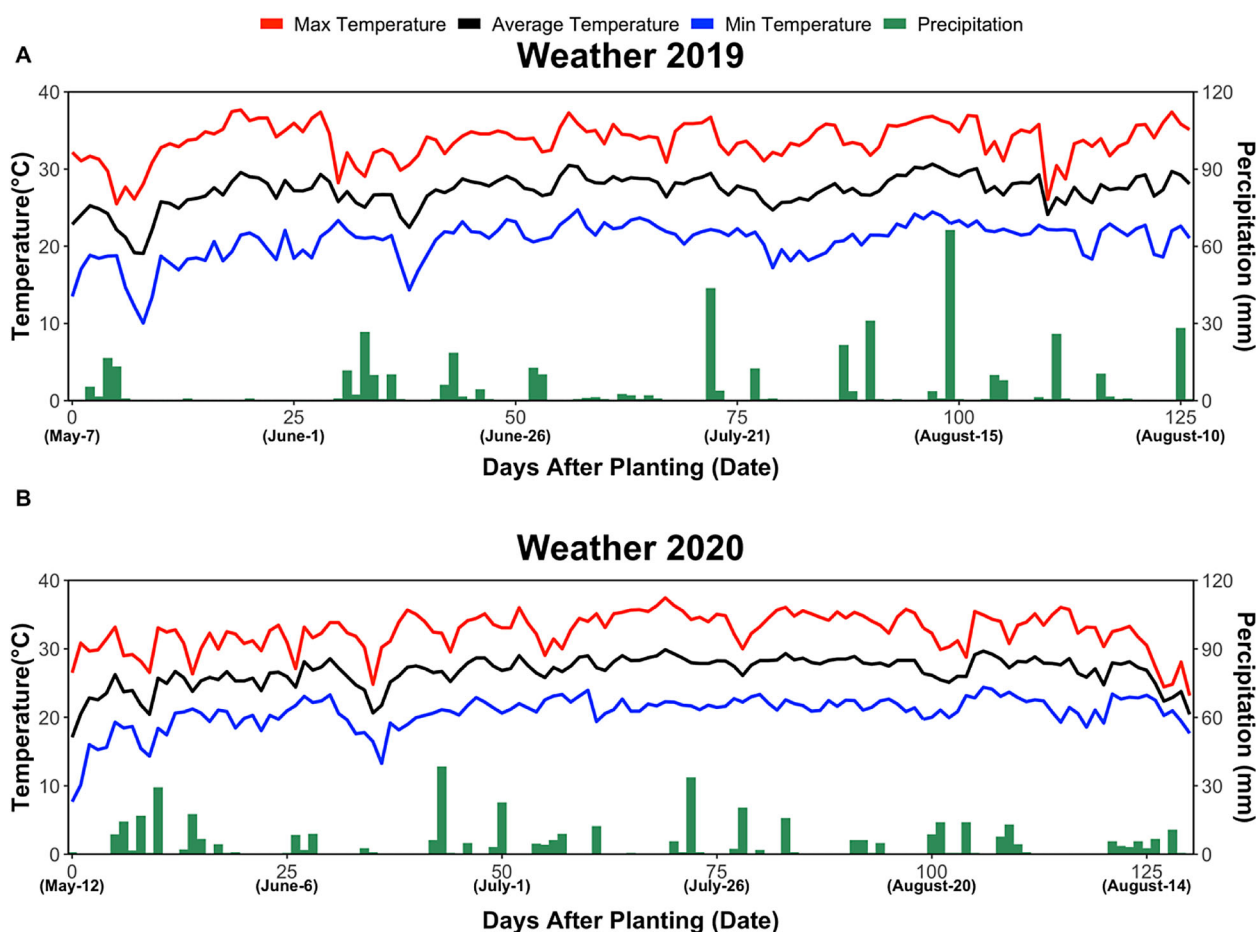
### Core Ideas

- Drought-tolerant peanut genotypes with high water use efficiency (WUE) and efficient use of water (EUW) were found.
- Genotypes PI 502120 and AU-NPL 17 were classified as high EUW (water spenders).
- Genotypes AU16-28 and Line-8 were classified as high WUE (water savers).
- Gas exchange was more effective than  $\Delta^{13}\text{C}$  to differentiate between WUE and EUW genotypes.

Although water spender genotypes have been described in other crops such as rice (*Oryza sativa* L.) (Kobata et al., 1996), wheat (*Triticum aestivum* L.) (Zhu et al., 2008), and common bean (*Phaseolus vulgaris* L.) (Sanz-Saez et al., 2019), there are no reports describing this drought-tolerant mechanism in peanuts and evaluating if this mechanism can be more advantageous than water savers in an environment such as the southeastern United States.

The most accurate way to calculate WUE and water use is the use of mini-lysimeters, which can measure the amount of water that is transpired by the plants during the growing season (Vadez & Ratnakumar, 2016). However, this method requires specialized facilities and is very labor intensive, which limits the number of genotypes that can be analyzed. As an alternative, researchers have used the carbon isotope discrimination ( $\Delta^{13}\text{C}$ ) technique to estimate WUE in many C3 crops (Buezo et al., 2019; Condon et al., 2002; Condon et al., 2006; Sanz-Saez et al., 2019), including peanut (Wright et al., 1994). In these experiments, Wright et al. (1994) showed that genotypes with lower  $\Delta^{13}\text{C}$  had limited transpiration and higher yield under terminal drought conditions, leading to a higher WUE (Condon et al., 2002; Condon et al., 2006). In contrast, water spenders genotypes keep stomata open during drought, using more water and continue the uptake of  $\text{CO}_2$  into the mesophyll, leading to increased  $\Delta^{13}\text{C}$  values. Therefore, the  $\Delta^{13}\text{C}$  technique can be used to distinguish between these two drought tolerance mechanisms (Polania et al., 2016; Sanz-Saez et al., 2019).

The disadvantage of using the carbon isotope discrimination technique is that it is time consuming to prepare the samples for analysis and the cost of the isotopic analysis. Other ways to differentiate between water saver and water spender genotypes could be to measure leaf gas exchange with a portable gas exchange analyzer and compare the photosynthesis and stomatal conductance ( $g_s$ ) of all the genotypes. In this case, water saver genotypes would show lower  $g_s$  than water spenders. In previous experiments this technique has been discarded as being not precise enough to separate



**FIGURE 1** Precipitation, maximum, minimum and mean day temperature recorded during the 2019 and 2020 growing season by a weather station located at the rain-out shelter facility at the USDA-ARS National Peanut Laboratory in Dawson, GA

between high and low WUE genotypes primarily due to the limited number of measurements, where only one or two measurements were taken during the experiments (Medrano et al., 2015; Polania et al., 2016; Sanz-Saez et al., 2019). However, this method has not been tested to separate between water saver and water spender genotypes using a more intense sampling schedule during the drought period.

The objectives of this study were to (a) screen peanut genotypes for water saver and water spender characteristics and (b) determine if frequent gas exchange measurements can be used in place of carbon isotope discrimination to differentiate between water saver and water spender genotypes.

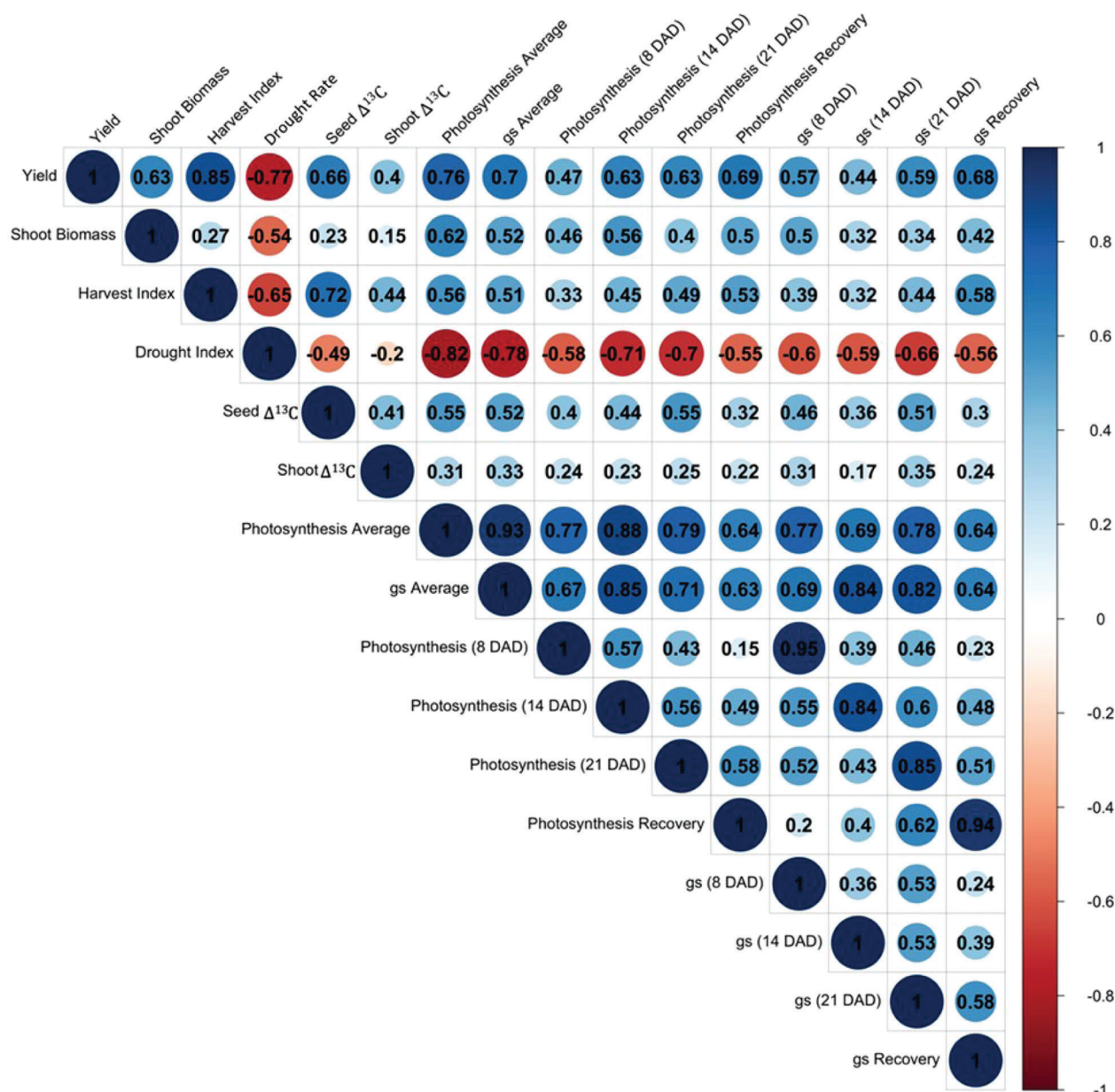
## 2 | MATERIALS AND METHODS

### 2.1 | Field site, experiment design, plant material, and crop management

This study was conducted in the 2019 and 2020 growing seasons in the rainout shelter facility at the USDA-ARS National Peanut Research Laboratory in Dawson, GA, which was

designed to prevent precipitation on plants during rain events (Blankenship et al., 1980). Each metal shelter covers a ground area of 5.5 m × 12.2 m and is connected to a rain detector (Agrowtek IR Digital Rain Sensor, Agroetek) that automatically closes when a drop of water touches the sensor. Drought treatments were implemented in July and August to simulate mid-season drought stress coinciding with the pod filling stage, as it is the most common time for drought in the southeastern United States. The weather data for both years was collected using an on-site weather station (Figure 1). The soil in the rainout shelters was a Tifton sandy loam (fine-loamy, kaolinitic, thermic Plinthic Kandiudults).

In 2019, 36 peanut genotypes previously described as drought-tolerant and/or -sensitive were planted on 7 May in six rainout shelters (Table 1). Of those, five shelters were treated as drought stress plots and one shelter was maintained as a well-watered control. Each genotype was hand planted in each rainout shelter in a 1.2-m single row with 0.9 m of separation between rows and a 0.6-m alley to avoid contamination between genotypes in the same row. All genotypes were planted in each rainout shelter, ending up with five replications of drought-stressed plants and one replication for



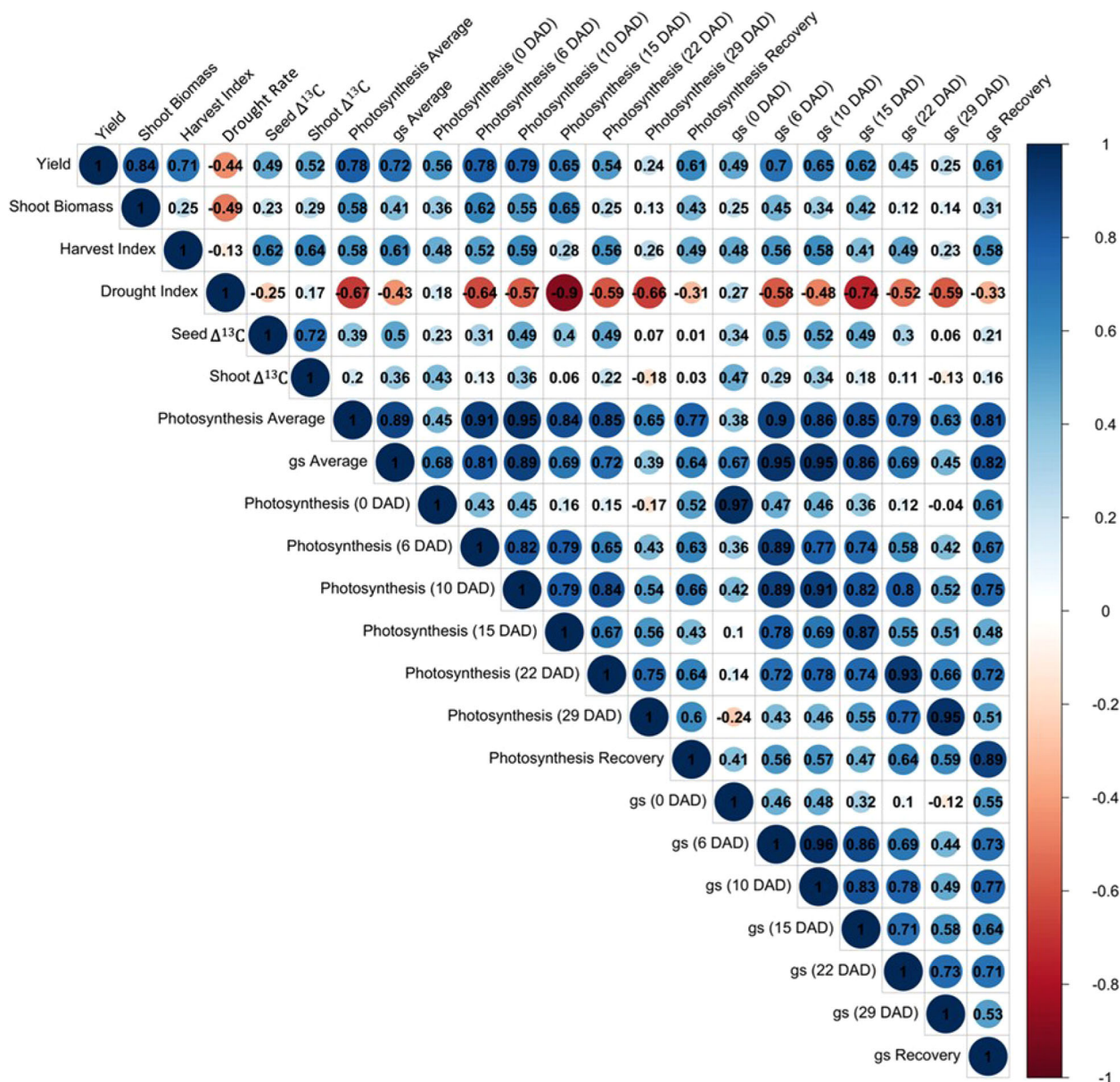
**FIGURE 2** Correlation matrix of all measured parameters in 2019. List of parameters measured: Yield, shoot biomass, HI (harvest index), drought rating, seed  $\Delta^{13}\text{C}$ , shoot  $\Delta^{13}\text{C}$ , photosynthesis average, stomatal conductance ( $g_s$ ) average, photosynthesis 8 days after drought (DAD), photosynthesis 14 DAD, photosynthesis 21 DAD photosynthesis recovery,  $g_s$  8 DAD,  $g_s$  14 DAD,  $g_s$  21 DAS,  $g_s$  recovery. The size and number of the circle indicate the strength of the relationship and correlation coefficient ( $r$ ). Blue color indicates a positive relationship and red indicates a negative relationship between parameters

irrigated plants. In 2020, 18 peanut genotypes subselected from the 2019 experiment (except AP-3, which was added as a drought-sensitive check), were planted on 12 May in four rainout shelters (Table 1). Three shelters were treated as drought plots, and one shelter was maintained as a well-watered control. Each genotype was hand planted in each rainout shelter in a 2.4-m single row separated by a 0.6-m alley to avoid contamination between genotypes. All genotypes were planted in each rainout shelter resulting in three

replications of drought-stressed plants and one replication for irrigated plants.

Each shelter had a set of Water Mark soil moisture sensors (Irrometer) placed in the center of the shelter at a depth of 10 and 20 cm. Before the drought treatment was imposed and for the irrigated treatment during the drought, each time that the mean of the two soil moisture sensors was below  $-60$  kPa, the plots were irrigated with 12.7 mm of water. The drought treatment in 2019 and 2020 started 60 (7 July) and





**FIGURE 3** Correlation matrix of all measured parameters in 2020. List of parameters measured: Yield, shoot biomass, HI (harvest index), drought rating, seed  $\Delta^{13}\text{C}$ , shoot  $\Delta^{13}\text{C}$ , photosynthesis average, stomatal conductance ( $g_s$ ) average, photosynthesis 0 days after drought (DAD), photosynthesis 6 DAD, photosynthesis 10 DAD photosynthesis 15 DAD, photosynthesis 22 DAS, photosynthesis 29 DAS, photosynthesis recovery,  $g_s$  0 DAD,  $g_s$  6 DAD,  $g_s$  10 DAS,  $g_s$  15 DAD,  $g_s$  22 DAS,  $g_s$  29 DAS,  $g_s$  recovery. The size and number of the circle indicate the strength of the relationship and correlation coefficient ( $r$ ). Blue color indicates a positive relationship and red indicates a negative relationship between parameters

70 (21 July) days after planting, respectively. In 2019, the imposed drought lasted for 4 wk (28 d) until 3 August, then the plants were watered again with 12.7 mm of water and left to mature until harvest on 10 September. In 2020, the imposed drought lasted for 5 wk (35 d) until 25 August, due to mild temperatures that delayed the onset of visual drought symptoms. After 25 August, plots were irrigated with 12.7 mm of water and left to mature until harvest on 19 September. During the recovery period, the shelters were not active, and irrigation was activated when the soil moisture sensors

showed values lower than  $-60$  kPa. At harvest, all tested entries were considered to have similar maturity requirements, thus harvest time was determined by the hull scrape method (Williams & Drexler, 1981) based on the genotype Georgia Green in each shelter. Peanuts were harvested with a plot thresher (Kingaroy Engineering Works, Kingaroy). During the experiment, all agronomic management practices related to fertilization and weed and pest control were applied according to University of Georgia best management practices for peanut.

**TABLE 1** Genotype and landrace names used in this experiment in 2019 and 2020 based on drought tolerance or sensitivity reported on publications or by previous field-based experiments in Auburn University Breeding Program

Cultivar	Selection characteristic	Experiment year		Reported in publication or personal communication by breeder
		2019	2020	
PI 502120	Drought tolerant	X	X	Charles Chen (personal communication [PC])
PI 493329	Drought tolerant	X	X	Charles Chen (PC)
TifNV-High O/L	Drought tolerant	X	X	Tillman (2019)
Tifrunner	Drought tolerant	X	X	Holbrook and Culbreath (2007)
Line-8	Drought tolerant	X	X	Charles Chen (PC)
G06G	Drought tolerant	X	X	Tillman (2019)
C76-16	Drought tolerant	X	X	Dang et al. (2012)
AU-NPL 17	Drought tolerant	X	X	Tillman (2019)
Line-4	Drought tolerant	X	X	Charles Chen (PC)
AU16-28	Drought tolerant	X	X	Charles Chen (PC)
AU18-35	Drought tolerant		X	Charles Chen (PC)
SPT06-6	Drought tolerant	X		Charles Chen (PC)
PI 196635	Drought tolerant	X		Charles Chen (PC)
PI 576636	Moderate drought tolerant	X		Rosas-Anderson et al. (2014)
Ga Green	Moderate drought tolerant	X	X	Dang et al. (2013)
Ga HI O/L	Moderate drought tolerant	X		Anco and Thomas (2021)
PI 497648	Moderate drought tolerant	X		Charles Chen (PC)
Line-2	Moderate drought tolerant	X		Charles Chen (PC)
PI 370331	Moderate drought tolerant	X		Charles Chen (PC)
PI 337406	Moderate drought tolerant	X		Charles Chen (PC)
PI 268996	Moderate drought tolerant	X		Charles Chen (PC)
PI 290566	Moderate drought tolerant	X		Kottapalli et al. (2009)
CG7-A	Moderate drought tolerant	X		Subramanian et al. (2000)
AT 3085RO	Drought susceptible	X		Anco and Thomas (2021)
PI 290594	Drought susceptible	X		Charles Chen (PC)
PI 155107	Drought susceptible	X		Charles Chen (PC)
PI 493938	Drought susceptible	X		Charles Chen (PC)
PI 339960	Drought susceptible	X	X	Charles Chen (PC)
PI 290560	Drought susceptible	X	X	Charles Chen (PC)
PI 274193	Drought susceptible	X		Kottapalli et al. (2009)
PI 390428	Drought susceptible	X	X	Charles Chen (PC)
PI 442768	Drought susceptible	X		Charles Chen (PC)
PI 493581	Drought susceptible	X		Charles Chen (PC)
PI 268755	Drought susceptible	X	X	Charles Chen (PC)
PI 288210	Drought susceptible	X		Charles Chen (PC)
PI 372305	Drought susceptible	X		Kottapalli et al. (2009)
PI 325943	Drought susceptible	X	X	Charles Chen (PC)
AP-3	Drought susceptible		X	Dang et al. (2012)

## 2.2 | Physiological trait measurements

Mid-day leaf photosynthesis and  $g_s$  were measured on the youngest fully expanded tetrafoliate in the top of the main

stem between 10:30 a.m. and 2 p.m., using two or three sets of the LI-6400XT Portable Photosynthesis System (LI-COR Biosciences). In 2019, measurements were performed on 8, 14, and 21 d after the start of drought (DAD), as well as

2 weeks after the end of the drought period (during recovery). In 2019, genotype one gas exchange measurement per plot was performed due to the high number of genotypes and replications in the experiment. In 2020, measurements were performed on 0, 6, 10, 15, 22, 29 DAD, as well as 2 weeks after the end of the drought period (during recovery). In 2020, two measurements were taken in each plot and averaged to obtain the mean plot values.

### 2.2.1 | Yield, HI, drought rating, and carbon isotope calculations

Harvest was performed by hand inversion of plants using shovels on 10 Sept. 2019 and 19 Sept. 2020. Three representative plants per plot were separated for HI and stable isotope analysis, then all plants were threshed using a small plot thresher. The representative plants were separated into pods and shoot biomass and dried for at least 72 h at 60 °C then weighed to record dry weight. Harvest index was calculated as:

$$HI = \frac{\text{pods DW}}{\text{pods DW} + \text{shoot DW}}$$

where HI is the harvest index and DW is dry weight of each organ. The pods collected from the peanut thresher were dried at 40 °C until they reached 0% moisture, and the pod weight from the three representative plants used to calculate the HI was added to the total pod weight of each plot to calculate yield in kg ha<sup>-1</sup>.

Drought rating was estimated by visual observation of the canopy the last day of the drought imposition. The visual rating varied from 1 (canopies not showing any symptoms of drought) to 5 (plants with all leaves wilted, brown and the majority fallen).

Dry seeds and shoot biomass samples were ground separately to pass a 1-mm sieve, weighed in tin capsules, and sent to the University of California Davis Stable Isotopes Facility for carbon isotope analysis using an isotope ratio mass spectrometer (IsoPrime, Elementar) connected to an element analyzer (EA3000, EuroVector).

The ratio (R) of <sup>13</sup>C/<sup>12</sup>C was shown as δ<sup>13</sup>C (‰), indicating the C isotope composition relative to Vienna Pee Dee Belemnite calcium carbonate:

$$\delta^{13}\text{C} = (R_{\text{samples}}/R_{\text{standard}}) - 1$$

δ<sup>13</sup>C (‰) values were standardized to C isotope discrimination (Δ<sup>13</sup>C, ‰) and values were calculated by:

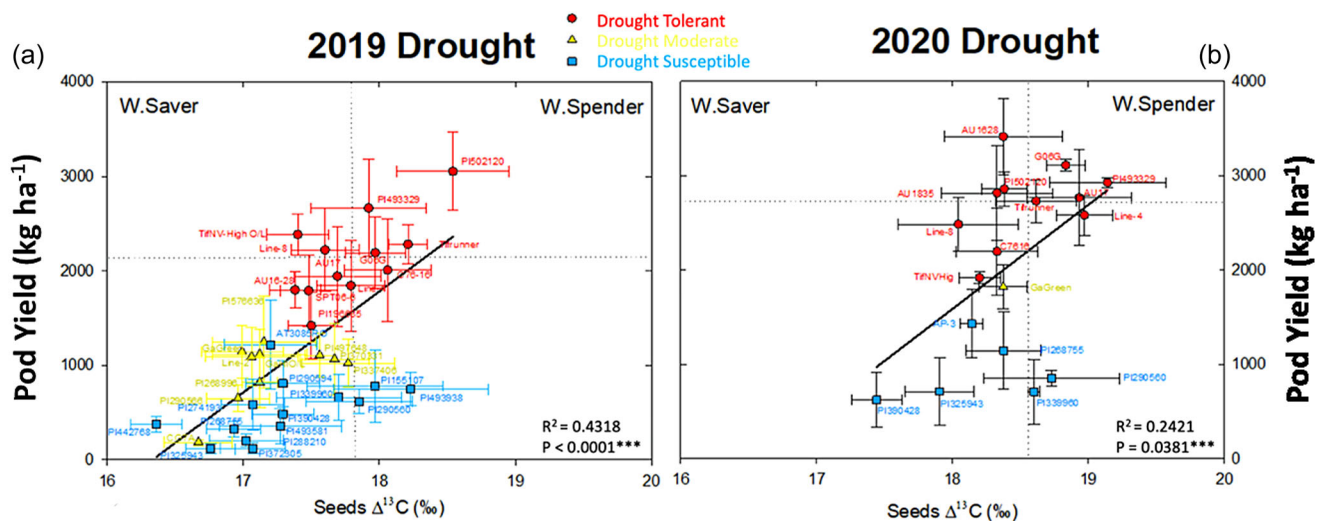
$$\Delta^{13}\text{C} (\text{‰}) = \left[ \frac{\delta^{13}\text{C}_{\text{atm}} - \delta^{13}\text{C}_{\text{sample}}}{1 + \left( \frac{\delta^{13}\text{C}_{\text{sample}}}{1,000} \right)} \right]$$

where δ<sup>13</sup>C<sub>atm</sub> is the C isotope composition of atmospheric CO<sub>2</sub> (−8‰; Farquhar et al., 1989), and δ<sup>13</sup>C<sub>sample</sub> is the C isotope composition of the plant sample (shoot biomass or seed). Seed and shoot biomass carbon isotope discrimination were reported as seed Δ<sup>13</sup>C and shoot Δ<sup>13</sup>C, respectively. A plot of yield vs Δ<sup>13</sup>C (Figure 4) was developed to differentiate between water spenders and savers (Polania et al., 2016; Sanz-Saez et al., 2019). The same approach was used for the average of photosynthesis and g<sub>s</sub> data (Figure 5). Genotypes that had higher Δ<sup>13</sup>C and g<sub>s</sub> than the mean of all the genotypes under drought were considered water spenders, whereas the ones that showed lower values than the mean were considered water saver genotypes (Polania et al., 2016; Sanz-Saez et al., 2019).

### 2.3 | Statistical analysis

Due to the use of inconsistent number of genotypes and different measurements over 2 yr, all the data was analyzed separately by year. As the irrigated treatment only consisted of one replicate, there was a statistical comparison of means that included all of the genotypes between drought and irrigated treatments. However, to corroborate the negative effects of drought, a one-way analysis of the variance (ANOVA) was performed. In this analysis, the mean of each genotype under drought was considered as one replication, whereas the single value of each genotype was used as a replication in the well-watered treatment. The analysis of the drought effect was only used to ensure that the drought treatment had a significant negative effect on the growth and physiology of the plants. To determine differences between genotypes under drought for each measured parameter, a mixed model ANOVA was conducted using PROC GLIMMIX in SAS 9.4 (SAS Institute) with genotypes as a fixed effect and replication as a random effect.

As physiological parameters (photosynthesis and g<sub>s</sub>) under drought were measured at different times during the drought period, a repeated measures mix model ANOVA PROC MIXED (SAS 9.4; SAS Institute) with genotypes as the fixed



**FIGURE 4** Correlation between yield and seed  $\Delta^{13}C$  in 2019 (A) and 2020 (B). Genotypes were divided into water saver and water spender categories according to the classification used by Sanz-Saez et al. (2019). Black trend lines represent a significant correlation ( $R^2$  and  $p$  values) including all the genotypes. Grey trend line indicates significant correlation of only classified drought-tolerant genotypes (red circles). Red Circles indicate drought-tolerant genotypes, yellow triangles indicate moderate drought-tolerant genotypes and blue squares indicate drought susceptible genotypes

effect, DAD as repeated measurements, and block (shelter) as the random effect was performed to analyze the effect of DAD and genotypes over measured parameters.

Correlation between all measured parameters was analyzed using PROC CORR (SAS 9.4; SAS Institute). To show correlations in a more visual display, a correlation matrix was obtained using CORR PLOT in R.

### 3 | RESULTS

#### 3.1 | Yield and agronomic traits

In 2019 and 2020, the implementation of mid-season drought using rainout shelters was effective at reducing yield ( $P < .0001$  and  $P = .0047$ , respectively), as the mean of all the genotypes under drought (1,180 and 2,063 kg ha<sup>-1</sup>) was significantly lower than the mean under irrigation (2,777 and 3,183 kg ha<sup>-1</sup>; Tables 2 and 3, respectively). The yield under drought in 2020 was nearly double the yield in 2019 (Tables 2 and 3), probably due to more extreme temperatures in 2019 that accentuated the drought stress. In addition, in both years, pod yield under mid-season drought conditions was significantly different between genotypes (Tables 2 and 3). In 2019, some of the previously identified drought-tolerant genotypes (PI 502120, PI 493329, TifNV-High O/L, Tifrunner, Line-8, and G06G) showed the highest yields with significant differences compared with moderate tolerant and drought-sensitive genotypes but with no difference between the six of them (Table 2). The average yield of these top six yielding genotypes (2,466 kg ha<sup>-1</sup>) was very similar to the average yield of

one replicate of all the genotypes under irrigated conditions (2,777 kg ha<sup>-1</sup>). The most drought susceptible genotype, PI 372305, produced 117 kg ha<sup>-1</sup>, which was 27 times lower than the highest yielding genotype PI 502120. In 2020, the high-yielding genotypes under drought, AU16-28, G06G, PI 493329, PI 502120, AU18-35, AU-NPL 17, Tifrunner, and Line-4 showed higher yields than the drought-sensitive ones, while they showed similar values (2,901 kg ha<sup>-1</sup>) to the average of all the genotypes grown under well-watered conditions (3,181 kg ha<sup>-1</sup>) (Table 3). The genotype PI 390428 was the most drought susceptible genotype producing 623.8 kg ha<sup>-1</sup> which was 5.5 times lower than the highest yielding genotype, AU16-28 (Table 3).

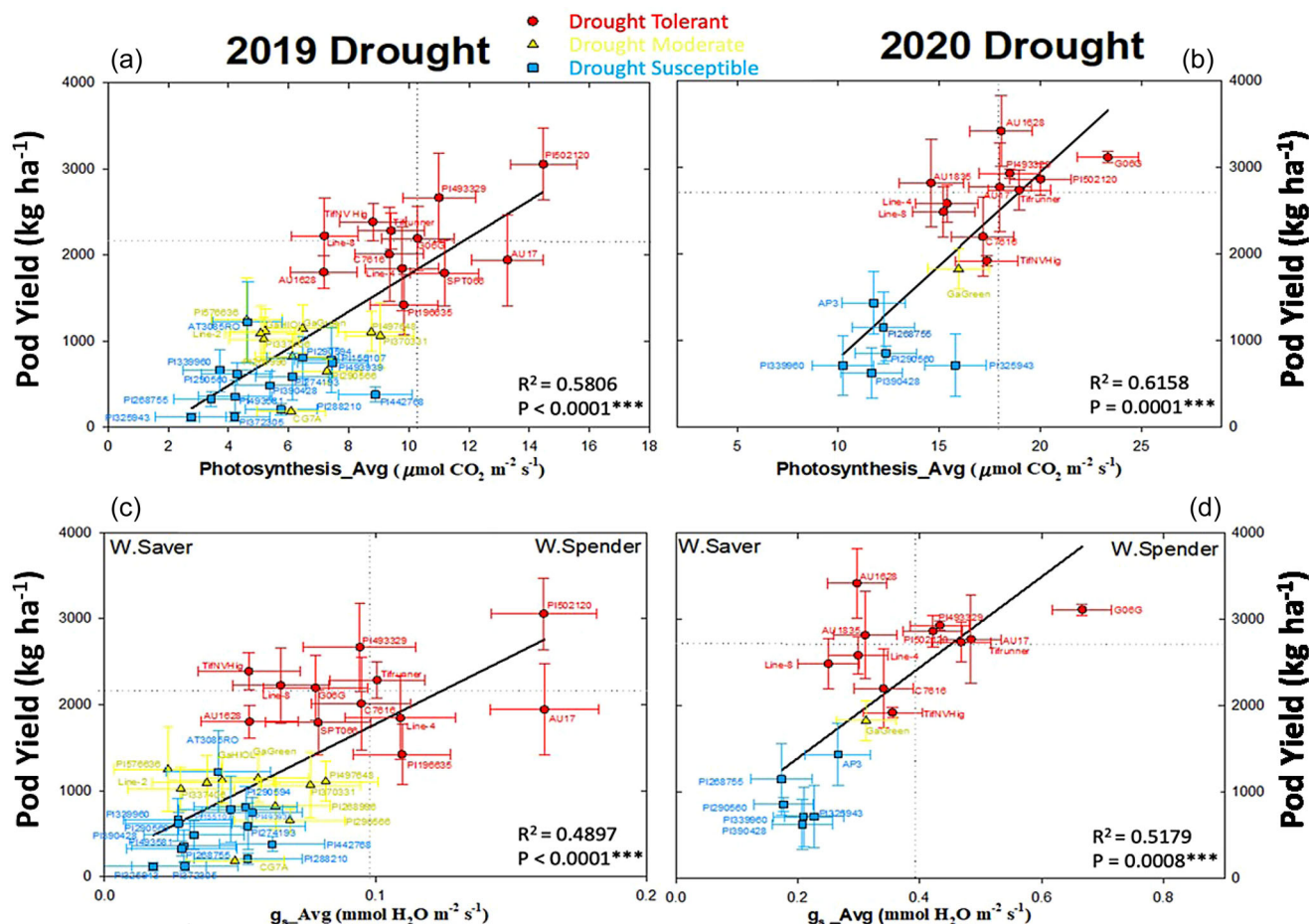
In 2019 and 2020, the mean shoot biomass of genotypes grown under drought was significantly lower than the biomass of those grown under irrigated conditions (Tables 2 and 3). In addition, shoot biomass under drought was significantly different among genotypes in both years (Tables 2 and 3). Most drought-tolerant genotypes showed higher shoot biomass than the drought-sensitive ones (Tables 2 and 3). However, in 2019, some low-yielding genotypes such as PI 274193 and PI 290594 showed a high shoot biomass of 4,411 kg ha<sup>-1</sup> and 4,255 kg ha<sup>-1</sup>, respectively, which was similar to the shoot biomass of drought-tolerant genotypes TifNV-High O/L and SPT06-6 (Table 2). Similar trends were observed in 2020 for the low yielding genotype PI 325943 (Table 3). This disparity is shown in the low HI for all of the low yielding genotypes that had high shoot biomass accumulation (Table 2 and 3) and this significantly affected the correlation observed between yield and shoot biomass in 2019 ( $R^2 = 0.39$ ;  $P \leq .0001$ ) and 2020 ( $R^2 = 0.69$ ;  $P \leq .0001$ ) (Figure 2; 3).



**TABLE 2** Yield, shoot biomass, harvest index (HI), and drought rating mean values  $\pm$  standard error of 36 genotypes grown under drought conditions in 2019

Genotype	Yield	Shoot biomass	HI	Drought rating
	kg ha <sup>-1</sup>			
PI 502120 (DT)	3,055.2 $\pm$ 414.3 a	3,223.5 $\pm$ 466.7 abcdefg	0.49 $\pm$ 0.01 a	2 $\pm$ 0.4 h
PI 493329 (DT)	2,666.5 $\pm$ 513 ab	4,007.8 $\pm$ 816.5 abcd	0.4 $\pm$ 0.03 abc	3 $\pm$ 0.7 defgh
TifNV-High O/L (DT)	2,385.6 $\pm$ 217.2 abc	4,756.3 $\pm$ 548.4 a	0.34 $\pm$ 0.03 bcdefghi	3 $\pm$ 0.4 defgh
Tifrunner (DT)	2,281.9 $\pm$ 210.6 abcd	4,260.8 $\pm$ 1,037.8 abc	0.37 $\pm$ 0.04 bcdef	2.7 $\pm$ 0.5 efgh
Line-8 (DT)	2,221.1 $\pm$ 440 abcd	3,636.7 $\pm$ 422.4 abcde	0.37 $\pm$ 0.02 bcdef	3.3 $\pm$ 0.6 bcdefg
G06G (DT)	2,192.2 $\pm$ 377 abcd	3,239.7 $\pm$ 472.8 abcdefg	0.4 $\pm$ 0.04 ab	3.5 $\pm$ 0.5 bcdef
C76-16 (DT)	2,009.6 $\pm$ 543.5 bcde	3,062.8 $\pm$ 588.8 abcdefg	0.39 $\pm$ 0.03 abcde	3 $\pm$ 0.6 defgh
AU-NPL17 (DT)	1,942.3 $\pm$ 530.4 bcdef	3,604.1 $\pm$ 461.4 abcde	0.33 $\pm$ 0.06 bcdefghi	2.1 $\pm$ 0.7 gh
Line-4 (DT)	1,843.5 $\pm$ 485.3 bcdefg	3,798.5 $\pm$ 1,215.7 abcde	0.34 $\pm$ 0.02 bcdefghi	3.4 $\pm$ 0.7 bcdef
AU16-28 (DT)	1,800.4 $\pm$ 189 bcdefg	2,911.4 $\pm$ 387 abcdefg	0.39 $\pm$ 0.01 abcd	3.1 $\pm$ 0.6 cdefgh
SPT06-6 (DT)	1,790.8 $\pm$ 380.6 cdefg	4,571.8 $\pm$ 661.4 a	0.28 $\pm$ 0.03 defghijk	2.5 $\pm$ 0.7 fgh
PI 196635 (DT)	1,421.3 $\pm$ 350.3 defgh	3,444.2 $\pm$ 795.1 abcdef	0.29 $\pm$ 0.04 cdefghijk	2.7 $\pm$ 0.4 efgh
PI 576636 (MDT)	1,247.6 $\pm$ 488.1 efghi	2,968.1 $\pm$ 641.2 abcdefg	0.28 $\pm$ 0.04 efghijk	3.3 $\pm$ 0.6 bcdefg
AT 3085RO (DS)	1,219.8 $\pm$ 472 efghi	3,242.7 $\pm$ 641.6 abcdefg	0.24 $\pm$ 0.05 mhiijkl	4.1 $\pm$ 0.5 abcd
Ga Green (MDT)	1,143.9 $\pm$ 277.2 efghij	2,032.3 $\pm$ 514.3 defg	0.36 $\pm$ 0.01 bcdefg	3.7 $\pm$ 0.3 bcdef
Ga HI O/L (MDT)	1,117.6 $\pm$ 261.2 fghij	2,367.8 $\pm$ 518.6 bcdefg	0.32 $\pm$ 0.06 bcdefghij	3.1 $\pm$ 0.5 cdefgh
PI 497648 (MDT)	1,106.2 $\pm$ 232.6 fghij	1,734.3 $\pm$ 201.3 efg	0.36 $\pm$ 0.04 bcdefg	3 $\pm$ 0.3 defgh
Line-2 (MDT)	1,090.6 $\pm$ 314.3 fghij	1,927.4 $\pm$ 469.1 defg	0.37 $\pm$ 0.07 bcdefg	3.9 $\pm$ 0.7 abcde
PI 3703 (MDT)31	1,064.1 $\pm$ 379.1 ghijk	3,124.5 $\pm$ 923.7 abcdefg	0.23 $\pm$ 0.04 mijklm	4.1 $\pm$ 0.3 abcd
PI 337406 (MDT)	1,021.2 $\pm$ 253.8 ghijkl	2,078.8 $\pm$ 634.8 cdefg	0.35 $\pm$ 0.02 bcdefgh	3.8 $\pm$ 0.3 abcde
PI 268996 (MDT)	819.7 $\pm$ 264.8 hijklm	3,175.6 $\pm$ 665.7 abcdefg	0.19 $\pm$ 0.04 mklno	3.7 $\pm$ 0.4 bcdef
PI 290594 (DS)	808.6 $\pm$ 242 hijklm	4,254.9 $\pm$ 2,483.2 abc	0.21 $\pm$ 0.05 mijklm	4.2 $\pm$ 0.3 abcd
PI 155107 (DS)	782.3 $\pm$ 381.9 hijklm	1,556 $\pm$ 556.1 efg	0.34 $\pm$ 0.04 bcdefghi	4.1 $\pm$ 0.4 abcd
PI 493938 (DS)	747.5 $\pm$ 174 hijklm	2,601 $\pm$ 770.3 abcdefg	0.28 $\pm$ 0.08 efghijk	3.4 $\pm$ 0.4 bcdef
PI 339960 (DS)	661.4 $\pm$ 244.4 hijklm	1,643.7 $\pm$ 499.3 efg	0.26 $\pm$ 0.05 ghijkl	4.3 $\pm$ 0.3 abc
PI 290566 (MDT)	651.3 $\pm$ 137.8 hijklm	2990.2 $\pm$ 1044.6 abcdefg	0.2 $\pm$ 0.03 mklno	4 $\pm$ 0.3 abcd
PI 290560 (DS)	618.8 $\pm$ 128.8 hijklm	1,763.2 $\pm$ 284.9 efg	0.27 $\pm$ 0.05 fghijkl	3.7 $\pm$ 0.3 bcdef
PI 274193 (DS)	584.6 $\pm$ 270.7 hijklm	4,411.1 $\pm$ 1,167.1 ab	0.12 $\pm$ 0.03 nop	4 $\pm$ 0.4 abcd
PI 390428 (DS)	484.5 $\pm$ 169.4 ijklm	2,644.8 $\pm$ 970.2 abcdefg	0.16 $\pm$ 0.02 mlnop	3.9 $\pm$ 0.5 abcde
PI 442768 (DS)	377.1 $\pm$ 87.3 ijklm	4,080.7 $\pm$ 1,201.5 abcd	0.1 $\pm$ 0.02 op	3.4 $\pm$ 0.2 bcdef
PI 493581 (DS)	355.6 $\pm$ 189.7 ijklm	1,246.7 $\pm$ 129.8 fg	0.19 $\pm$ 0.08 mklno	3.9 $\pm$ 0.4 abcde
PI 268755 (DS)	325.5 $\pm$ 83.3 jklm	1,387.6 $\pm$ 371.3 fg	0.2 $\pm$ 0.04 mklno	4.3 $\pm$ 0.3 abc
PI 288210 (DS)	205.7 $\pm$ 63.8 klm	1,660.6 $\pm$ 381.7 efg	0.12 $\pm$ 0.03 nop	4.1 $\pm$ 0.3 abcd
CG7-A (MDT)	183.7 $\pm$ 29.1 lm	1,294.5 $\pm$ 288.9 fg	0.13 $\pm$ 0.02 mnop	3.6 $\pm$ 0.2 bcdef
PI 372305 (DS)	121.1 $\pm$ 54.6 m	1,268.6 $\pm$ 366.5 fg	0.07 $\pm$ 0.02 p	4.5 $\pm$ 0.2 ab
PI 325943 (DS)	117.1 $\pm$ 49.1 m	940.5 $\pm$ 280.9 g	0.1 $\pm$ 0.03 op	5 $\pm$ 0 a
Genotype ( <i>p</i> value)	<.0001***	<.0001***	<.0001***	0.0009***
<b>Water stress</b>				
Water stress mean	1,179.6 b	2,803.1 b	0.27333 b	3.5389 a
Irrigated mean	2,777.2 a	4,570.8 a	0.36583 a	1 b
Water stress ( <i>p</i> value)	<.0001***	<.0001***	0.0008***	<.0001***

*Note.* Means followed by common letters were statistically different. The table shows the genotype effect (*p* value) in the plants grown under midseason drought conditions. The genotypes order was organized from higher to lower yield under drought. In the bottom of the table, the mean of all genotypes grown in one replicate under irrigated conditions and the mean of all genotypes under drought conditions was shown for comparison using a one-way ANOVA. Genotypes were grouped as drought tolerant (DT), moderate drought tolerant (MDT), and drought susceptible (DS) based on previous studies.



**FIGURE 5** Correlation between yield with average photosynthesis (A, B) and average stomatal conductance (C, D) in 2019 and 2020, respectively. For photosynthesis, and stomatal conductance, genotypes were divided into water saver and water spender categories according to the classification used by Sanz-Saez et al. (2019). Black trend lines represent a significant correlation ( $R^2$  and  $p$  values) including all the genotypes. Red Circles indicate drought-tolerant genotypes, yellow triangles indicate moderate drought-tolerant genotypes and blue squares indicate drought susceptible genotypes

In 2019, the HI was significantly decreased by drought (Table 2); however, in 2020 it was not affected (Table 3). In both years the HI under drought was significantly different among genotypes ( $P < .0001$ ). In 2019, previously identified drought-tolerant genotypes showed higher HI (0.36) than the drought-sensitive ones (0.19; Table 2). Within the drought-tolerant genotype group, genotype PI 502120 had the highest HI (0.49) followed by G06G and PI 493329 (HI = 0.4) (Table 2). PI 372305, a drought-sensitive genotype, showed the lowest HI, which was 0.07 under drought. However, not all drought-sensitive genotypes had a low HI. For example, PI 155107 showed a HI of 0.34 which was similar to the mean of the high-yielding genotypes (0.37; Table 2). In 2020, the previously identified drought-tolerant genotypes showed a higher HI (0.46) than the drought-sensitive ones (0.32), but the difference was lower than in 2019, probably due to the milder weather conditions (temperatures) in 2020. Among the drought-tolerant genotypes, G06G had the highest HI of 0.52,

followed by AU-NPL 17 (0.5) and C76-16 (0.5) (Table 3). PI 325943 showed the lowest HI under drought conditions (0.21). Harvest index was positively and significantly correlated with yield in 2019 ( $R^2 = 0.71$ ;  $P \leq .0001$ ; Figure 2) and 2020 ( $R^2 = 0.50$ ;  $P \leq .001$ ; Figure 3).

In 2019 and 2020, drought rating values were significantly different among genotypes (Tables 2 and 3, respectively). In both years, higher yielding genotypes (the drought-tolerant ones) had the lowest drought rating value in comparison with the drought-sensitive ones, resulting in a negative relationship between yield and drought rating in 2019 (Figure 2) and in 2020 (Figure 3). In 2019, PI 502120, a drought-tolerant genotype, showed the lowest drought rating, whereas PI 325943, a drought-sensitive genotype, showed the highest value (Table 2). In 2020, some of the high-yielding genotypes (e.g., AU16-28 and G06G) showed the lowest drought rating, whereas one of the lowest yielding genotypes (PI 325943) showed the highest drought rating of all genotypes (Table 3).

**TABLE 3** Yield, shoot biomass, harvest index (HI), and drought rating mean values  $\pm$  standard error of 18 genotypes grown under drought conditions in 2020

Genotype	Yield	Shoot biomass	HI	Drought rating
	kg ha <sup>-1</sup>			
AU16-28 (DT)	3,415.6 ± 408.4 a	4,746 ± 564.5 a	0.42 ± 0.02 bc	2 ± 0.6 de
G06G (DT)	3,111.9 ± 64.1 ab	2,915.5 ± 234.5 bcde	0.52 ± 0.02 a	2 ± 0.6 de
PI 493329 (DT)	2,927.7 ± 53.2 abc	3014.6 ± 116.8 bcde	0.49 ± 0.01 abc	2.7 ± 0.6 cde
PI 502120 (DT)	2,861.8 ± 181.4 abc	3,118.2 ± 133.8 bcd	0.48 ± 0.01 abc	2.3 ± 0.6 de
AU 18–35 (DT)	2,816.3 ± 504.3 abc	3,793.5 ± 489.1 ab	0.42 ± 0.01 abc	4.3 ± 0.6 abc
AU-NPL17 (DT)	2,767.8 ± 508.8 abcd	2,838.6± 586.2 bcdef	0.5 ± 0.01 ab	3 ± 0.6 bcde
Tifrunne (DT)	2,733.8 ± 230.3 abcd	3,351.1 ± 277.4 bc	0.45 ± 0.02 abc	2.5 ± 0.6 de
Line-4 (DT)	2,581.5 ± 211 abcde	3,936.1 ± 330.1 ab	0.4 ± 0.03 cd	3.2 ± 0.6 bcde
Line-8 (DT)	2,485.5 ± 287.9 bcde	3,093.1 ± 860.7 bcd	0.47 ± 0.05 abc	2.3 ± 0.6 de
C76-16 (DT)	2,197.4 ± 457.1 cdef	2,258.3 ± 599.5 cdefg	0.5 ± 0.01 ab	3.7 ± 0.6 abcd
TifNVHig (DT)	1,918.1 ± 60.6 defg	2,820.3 ± 410.3 bcdef	0.41 ± 0.03 bcd	2.7 ± 0.6 cde
Ga Green (MDT)	1,824.8± 232.5 efg	2,002.7 ± 242.7 defg	0.48 ± 0.02 abc	4.5 ± 0.6 ab
AP-3 (DS)	1,432.9 ± 360.3 fgh	2,107.1 ± 230.8 cdefg	0.4 ± 0.05 cd	5 ± 0.6 a
PI 268755 (DS)	1148.8 ± 413 gh	1,683.3 ± 642.9 efg	0.4 ± 0.05 cd	3.5 ± 0.6 abcde
PI 290560 (DS)	854 ± 85.3 h	1,067.6 ± 213.3 g	0.45 ± 0.02 abc	3.5 ± 0.6 abcde
PI 325943 (DS)	714.7 ± 356 h	2,539.6 ± 978 efg	0.21 ± 0.02 f	1.8 ± 0.6 e
PI 339960 (DS)	711.4 ± 343.5 h	1,501.8 ± 764 g	0.32 ± 0.07 de	3.7 ± 0.6 abcd
PI 390428 (DS)	623.8 ± 289.3 h	1,606.7 ± 288.4 fg	0.25 ± 0.08 ef	4.5 ± 0.6 ab
Genotype ( <i>p</i> value)	<.0001***	<.0001***	<.0001***	<.0001***
Water stress				
Water stress mean	2,062.64 b	2,688.57 b	0.42	3.18 a
Irrigated mean	3,183.15 a	3,578.76 a	0.46	1.00 b
Water stress ( <i>p</i> value)	0.0047**	0.0163*	0.21	<.0001***

Note. Means followed by common letters were statistically different. The table shows the genotype effect (*p* value) in the plants grown under mid-season drought conditions. The genotypes order was organized from higher to lower yield under drought. In the bottom of the table the mean of all genotypes grown in one replicate under irrigated conditions and the mean of all genotypes under drought conditions was shown for comparison using a one-way ANOVA. Genotypes were grouped as drought tolerant (DT), moderate drought tolerant (MDT) and drought susceptible (DS) based on previous studies.

### 3.2 | Photosynthetic and water related traits

In 2019, there was a significant genotype effect on photosynthesis ( $P \leq .0001$ ), but not for DAD ( $P = .633$ ); however, there was a significant interaction between genotypes and DAD ( $P = .027$ ). In 2020, photosynthesis showed a significant effect on genotypes ( $P \leq .0001$ ) and DAD ( $P \leq .0001$ ), but there was no interaction ( $P = .195$ ). In 2019,  $g_s$  was significantly affected by genotype ( $P \leq .0001$ ) but not by the DAD ( $P = .465$ ) or the interaction between genotypes and DAD ( $P = .230$ ). In 2020,  $g_s$  was significantly affected by the genotype ( $P \leq .0001$ ), DAD ( $P \leq .0001$ ), and the interaction effect ( $P \leq .0001$ ). In both years, photosynthesis and  $g_s$  tended to decrease as the DAD progressed, recovering slightly after the watering treatment was re-imposed (recovery) (Tables S1 and S3).

To determine which genotype was more drought-tolerant during the entire drought period, the photosynthesis as well as

the  $g_s$  values were averaged across the drought and recovery periods for each genotype and year. In 2019, genotypes PI 502120 and AU-NPL 17 had the highest mean photosynthesis in comparison with other drought-tolerant genotypes such as TifNV High O/L, Tifrunner, Line-8, C76-16, Line-4, and AU16-28 (Table 4). In contrast, drought-sensitive genotypes such as PI 325943, PI 372305, PI 268755, and PI 339960 showed some of the lowest average photosynthetic rates (Table 4). The average photosynthesis of all genotypes under drought conditions was lower than the well-watered genotypes in 2019 (Table 4). In 2020, all drought-tolerant genotypes showed higher photosynthesis than the drought-sensitive ones; the exceptions to this trend were the drought-tolerant genotype AU18-35, which was similar to the drought-sensitive genotype group, and the drought-sensitive genotype PI 325943, a genotype that was similar to the drought-tolerant genotype group (Table 5). Of the drought-tolerant genotypes, G06G showed the highest

**TABLE 4** Carbon isotope discrimination in seed (Seed  $\Delta^{13}\text{C}$ ), carbon isotope discrimination in shoot biomass (Shoot  $\Delta^{13}\text{C}$ ), average photosynthesis (A), and average stomatal conductance ( $g_s$ ) mean values  $\pm$  standard error of 36 genotypes grown under mid-season drought conditions in 2019

Genotype	$\Delta^{13}\text{C}$ seed	$\Delta^{13}\text{C}$ shoot	Avg. A	Avg. $g_s$
	‰	‰	$\mu\text{mol m}^{-2} \text{s}^{-1}$	$\text{mmol m}^{-2} \text{s}^{-1}$
PI 502120 (DT)	18.5 $\pm$ 0.4 a	20 $\pm$ 0.3	14.46 $\pm$ 1.11 a	0.16 $\pm$ 0.02 a
PI 493329 (DT)	17.9 $\pm$ 0.4 abcde	20 $\pm$ 0.1	11 $\pm$ 1.22 bc	0.09 $\pm$ 0.02 bcde
TifNV-High O/L (DT)	17.4 $\pm$ 0.2 cdefghijk	19.3 $\pm$ 0.2	8.8 $\pm$ 1.11 cdefghi	0.05 $\pm$ 0.02 defghij
Tifrunner (DT)	18.2 $\pm$ 0.1 ab	19.8 $\pm$ 0.2	9.41 $\pm$ 1.11 cdef	0.1 $\pm$ 0.02 bc
Line-8 (DT)	17.6 $\pm$ 0.2 bcdefghij	19.6 $\pm$ 0.1	7.19 $\pm$ 1.11 defghijk	0.06 $\pm$ 0.02 bcdefghij
G06G (DT)	18 $\pm$ 0.2 abcd	19.8 $\pm$ 0.2	10.29 $\pm$ 1.22 bcd	0.08 $\pm$ 0.02 bcdefgh
C76-16 (DT)	18.1 $\pm$ 0.3 abc	19.9 $\pm$ 0.2	9.34 $\pm$ 1.15 cdefg	0.09 $\pm$ 0.02 bcd
AU-NPL17 (DT)	17.7 $\pm$ 0.3 bcdefghi	19.1 $\pm$ 0.4	13.27 $\pm$ 1.19 ab	0.16 $\pm$ 0.02 a
Line-4 (DT)	17.8 $\pm$ 0.2 abcdefg	19.7 $\pm$ 0.3	9.77 $\pm$ 1.22 cde	0.11 $\pm$ 0.02 b
AU16-28 (DT)	17.4 $\pm$ 0.2 cdefghijk	19.3 $\pm$ 0.1	7.17 $\pm$ 1.11 defghijk	0.05 $\pm$ 0.02 defghij
SPT06-6 (DT)	17.5 $\pm$ 0.2 bcdefghijk	19.1 $\pm$ 0.2	11.18 $\pm$ 1.15 bc	0.08 $\pm$ 0.02 bcdefg
PI 196635 (DT)	17.5 $\pm$ 0.2 bcdefghij	19.3 $\pm$ 0.1	9.82 $\pm$ 1.12 bcd	0.11 $\pm$ 0.02 b
PI 576636 (MDT)	17.2 $\pm$ 0.4 efghijkl	18.8 $\pm$ 0.4	4.61 $\pm$ 1.18 jklm	0.02 $\pm$ 0.02 j
AT 3085RO (DS)	17.2 $\pm$ 0.3 defghijkl	19.3 $\pm$ 0.3	4.64 $\pm$ 1.15 jklm	0.04 $\pm$ 0.02 efghij
Ga Green (MDT)	17 $\pm$ 0.3 ghijkl	18.8 $\pm$ 0.3	6.47 $\pm$ 1.17 fghijkl	0.06 $\pm$ 0.02 cdefghij
Ga HI O/L (MDT)	17.1 $\pm$ 0.3 efghijkl	19.4 $\pm$ 0.2	5.22 $\pm$ 1.18 jklm	0.04 $\pm$ 0.02 efghij
PI 497648 (MDT)	17.6 $\pm$ 0.1 bcdefghij	19.8 $\pm$ 0.1	8.75 $\pm$ 1.11 cdefghi	0.08 $\pm$ 0.02 bcdef
Line-2 (MDT)	17.1 $\pm$ 0.3 fghijkl	19.2 $\pm$ 0.3	5.06 $\pm$ 1.18 jklm	0.04 $\pm$ 0.02 fghij
PI 370331 (MDT)	17.7 $\pm$ 0.2 bcdefghi	19.8 $\pm$ 0.4	9.04 $\pm$ 1.13 cdefgh	0.08 $\pm$ 0.02 bcdefghi
PI 337406 (MDT)	17.8 $\pm$ 0.3 abcdefgh	19.5 $\pm$ 0.2	5.18 $\pm$ 1.15 jklm	0.03 $\pm$ 0.02 hij
PI 268996 (MDT)	17.1 $\pm$ 0.1 efghijkl	19.2 $\pm$ 0.1	6.13 $\pm$ 1.22 ghijkl	0.06 $\pm$ 0.02 bcdefghij
PI 290594 (DS)	17.3 $\pm$ 0.3 cdefghijk	19.7 $\pm$ 0.2	6.47 $\pm$ 1.19 efghijkl	0.05 $\pm$ 0.02 defghij
PI 155107 (DS)	18 $\pm$ 0.5 abcde	19.6 $\pm$ 0.2	7.42 $\pm$ 1.22 defghijk	0.05 $\pm$ 0.02 efghij
PI 493938 (DS)	18.2 $\pm$ 0.6 ab	19.4 $\pm$ 0.5	7.46 $\pm$ 1.18 defghij	0.05 $\pm$ 0.02 cdefghij
PI 339960 (DS)	17.7 $\pm$ 0.5 bcdefghi	19.6 $\pm$ 0.2	3.71 $\pm$ 1.22 lm	0.03 $\pm$ 0.02 ij
PI 290566 (MDT)	17 $\pm$ 0.2 hijkl	19.3 $\pm$ 0.2	7.27 $\pm$ 1.13 defghijk	0.07 $\pm$ 0.02 bcdefghij
PI 290560 (DS)	17.8 $\pm$ 0.4 abcdef	19.5 $\pm$ 0.3	4.28 $\pm$ 1.18 jklm	0.03 $\pm$ 0.02 ij
PI 274193 (DS)	17.1 $\pm$ 0.2 efghijkl	19.3 $\pm$ 0.3	6.13 $\pm$ 1.22 ghijkl	0.05 $\pm$ 0.02 defghij
PI 390428 (DS)	17.3 $\pm$ 0.2 cdefghijk	15.5 $\pm$ 0.3	5.38 $\pm$ 1.17 jklm	0.03 $\pm$ 0.02 fghij
PI 442768 (DS)	16.4 $\pm$ 0.2 L	18.8 $\pm$ 0.1	8.89 $\pm$ 1.23 cdefghi	0.06 $\pm$ 0.02 bcdefghij
PI 493581 (DS)	17.3 $\pm$ 0.5 cdefghijk	18.9 $\pm$ 0.7	4.22 $\pm$ 1.22 jklm	0.03 $\pm$ 0.02 fghij
PI 268755 (DS)	16.9 $\pm$ 0.2 ijkl	19.3 $\pm$ 0.2	3.42 $\pm$ 1.26 lm	0.03 $\pm$ 0.02 fghij
PI 288210 (DS)	17 $\pm$ 0.3 ghijkl	18.7 $\pm$ 0.2	5.74 $\pm$ 1.19 ijklm	0.05 $\pm$ 0.02 defghij
CG7-A (MDT)	16.7 $\pm$ 0.2 kl	18.9 $\pm$ 0.1	6.08 $\pm$ 1.13 hijkl	0.05 $\pm$ 0.02 efghij
PI 372305 (DS)	17.1 $\pm$ 0.2 fghijkl	19.1 $\pm$ 0.1	4.21 $\pm$ 1.18 klm	0.03 $\pm$ 0.02 fghij
PI 325943 (DS)	16.8 $\pm$ 0.2 jkl	19.1 $\pm$ 0.3	2.76 $\pm$ 1.17 m	0.02 $\pm$ 0.02 j
Genotype ( <i>p</i> value)	<.0001***	0.3637	<.0001***	<.0001***
<b>Water stress</b>				
Water stress mean	17.43 b	19.2619 b	7.20 b	0.06 b
Irrigated mean	18.53 a	19.9794 a	13.72 a	0.15 a
Water stress ( <i>p</i> value)	<.0001***	<.0001***	<.0001***	<.0001***

Note. Means followed by common letters were statistically different. The table shows the genotype effect (*p* value) in the plants grown under mid-season drought conditions. The genotypes order was organized from higher to lower yield under drought. In the bottom of the table the mean of all genotypes grown in one replicate under irrigated conditions and the mean of all genotypes under drought conditions was shown for comparison using a one-way ANOVA. Genotypes were grouped as drought tolerant (DT), moderate drought tolerant (MDT) and drought susceptible (DS) based on previous studies.



**TABLE 5** Carbon isotope discrimination in seed (Seed  $\Delta^{13}\text{C}$ ), carbon isotope discrimination in shoot biomass (Shoot  $\Delta^{13}\text{C}$ ), average photosynthesis (A), and average stomatal conductance ( $g_s$ ) mean values  $\pm$  standard error of 18 genotypes grown under mid-season drought conditions in 2020

Genotype	$\Delta^{13}\text{C}$ seed	$\Delta^{13}\text{C}$ shoot	Avg. A	Avg. $g_s$
	‰		$\mu\text{mol m}^{-2} \text{s}^{-1}$	$\text{mmol m}^{-2} \text{s}^{-1}$
AU 16–28 (DT)	18.4 $\pm$ 0.4 abc	19.9 $\pm$ 0.2 abc	18.06 $\pm$ 1.54 bcde	0.3 $\pm$ 0.05 fgh
G06G (DT)	18.8 $\pm$ 0.1 abc	20 $\pm$ 0.1 ab	23.35 $\pm$ 1.54 a	0.67 $\pm$ 0.05 a
PI 493329 (DT)	19.1 $\pm$ 0.4 a	20 $\pm$ 0.3 abc	18.49 $\pm$ 1.54 bcd	0.43 $\pm$ 0.05 bcd
PI 502120 (DT)	18.4 $\pm$ 0.2 abc	19.7 $\pm$ 0.2 bc	20.02 $\pm$ 1.54 b	0.42 $\pm$ 0.05 bcde
AU 18–35 (DT)	18.3 $\pm$ 0.4 abc	19.8 $\pm$ 0.3 abc	14.59 $\pm$ 1.59 fghi	0.31 $\pm$ 0.05 efgh
AU-NPL17 (DT)	18.9 $\pm$ 0.4 ab	20 $\pm$ 0.1 abc	18 $\pm$ 1.54 bcde	0.48 $\pm$ 0.05 b
Tifrunne (DT)	18.6 $\pm$ 0.3 abc	20 $\pm$ 0.2 abc	18.97 $\pm$ 1.54 bc	0.47 $\pm$ 0.05 bc
Line-4 (DT)	19 $\pm$ 0.2 ab	20.5 $\pm$ 0.2 a	15.39 $\pm$ 1.54 efg	0.3 $\pm$ 0.05 fgh
Line-8 (DT)	18 $\pm$ 0.4 cd	19.7 $\pm$ 0.1 bc	15.21 $\pm$ 1.54 efgh	0.25 $\pm$ 0.05 fghi
C76-16 (DT)	18.3 $\pm$ 0.3 abc	19.9 $\pm$ 0.1 abc	17.16 $\pm$ 1.54 bcdef	0.34 $\pm$ 0.05 defg
TifNVHig (DT)	18.2 $\pm$ 0.2 bcd	19.4 $\pm$ 0.1 bc	17.36 $\pm$ 1.54 bcdef	0.36 $\pm$ 0.05 cdef
Ga Green (MDT)	18.4 $\pm$ 0.2 abc	20.2 $\pm$ 0.3 ab	15.96 $\pm$ 1.54 cdef	0.31 $\pm$ 0.05 efgh
AP-3 (DS)	18.1 $\pm$ 0.1 bcd	19.9 $\pm$ 0.2 abc	11.76 $\pm$ 1.57 ij	0.27 $\pm$ 0.05 fghi
PI 268755 (DS)	18.4 $\pm$ 0.3 abc	19.6 $\pm$ 0.1 bc	12.23 $\pm$ 1.57 hij	0.17 $\pm$ 0.05 i
PI 290560 (DS)	18.7 $\pm$ 0.5 abc	19.8 $\pm$ 0.5 abc	12.36 $\pm$ 1.54 ghij	0.18 $\pm$ 0.05 i
PI 325943 (DS)	17.9 $\pm$ 0.3 bcd	18.9 $\pm$ 0.3 d	15.8 $\pm$ 1.54 def	0.23 $\pm$ 0.05 ghi
PI 339960 (DS)	18.6 $\pm$ 0 abc	19.9 $\pm$ 0.4 abc	10.22 $\pm$ 1.54 j	0.21 $\pm$ 0.05 hi
PI 390428 (DS)	17.4 $\pm$ 0.2 d	19.3 $\pm$ 0.4 cd	11.65 $\pm$ 1.54 ij	0.21 $\pm$ 0.05 hi
Genotype ( <i>p</i> value)	0.078	0.037*	<.0001***	<.0001***
<b>Water stress</b>				
Water stress mean	18.43 b	19.80 b	15.91 b	0.33 b
Irrigated mean	20.05 a	20.30 a	24.81 a	0.65 a
Water stress ( <i>p</i> value)	<.0001***	<.0008**	<.0001***	<.0001***

Note. Means followed by common letters were statistically different. The table shows the genotype effect (*p* value) in the plants grown under mid-season drought conditions. The genotypes order was organized from higher to lower yield under drought. In the bottom of the table the mean of all genotypes grown in one replicate under irrigated conditions and the mean of all genotypes under drought conditions was shown for comparison using a one-way ANOVA. Genotypes were grouped as drought tolerant (DT), moderate drought tolerant (MDT) and drought susceptible (DS) based on previous studies.

photosynthesis of all, and PI 339960 showed the lowest photosynthesis among the drought-sensitive ones. The mean photosynthesis across all genotypes under drought was significantly lower than that under irrigation (Table 5), confirming that the rainout shelters were successful at imposing drought and reducing gas exchange. All photosynthesis measurements were positively correlated with yield (Figures 2 and 3), but the average photosynthesis of all measurements performed during the drought period had the highest *r* value (0.76 in 2019 and 0.78 in 2020).

In both years, the mean  $g_s$  of genotypes grown under drought conditions was lower than the mean of genotypes under irrigated conditions (Table 4 and 5). In 2019, following a similar trend to photosynthetic data, genotypes PI 502120 and AU-NPL 17 maintained the highest  $g_s$  over the season, in comparison with other drought-tolerant genotypes, such as PI 493329, Tif-runner, C76-16, and Line-4. However, all of these genotypes showed higher  $g_s$  than the drought-sensitive

ones such as PI 337406, PI 339960, PI 290560, PI 390428, PI 493581, PI 268755, PI 372305, and PI 325943 (Table 4). Among the drought-tolerant genotypes, TifNV High O/L, Line-8, and AU16-28 showed the lowest  $g_s$  which was similar to drought-sensitive ones (Table 4). In 2020, when the genotype values were averaged across the drought and recovery periods, G06G had the highest  $g_s$  under drought and PI 268755 had the lowest  $g_s$  value (Figure 5D). The lowest  $g_s$  observed on PI 268755 (0.17  $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ ) under drought in 2020 was very similar to the highest  $g_s$  observed under drought in 2019 for PI 502120 (0.16  $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ ), which showed that the drought stress in 2019 was more severe than in 2020 as it led to lower  $g_s$  values. Among the high-yielding genotypes of 2020, AU16-28 and Line-8 showed the lowest  $g_s$ , which was similar to some of the drought-sensitive genotypes (e.g., PI 325943, PI 339060, and PI 390428), as well as in 2019 (Table 4 and 5). In both years,  $g_s$  under drought was always positively correlated with

yield, but the highest correlation corresponded to the average  $g_s$  of all measurements performed during the drought period ( $r = 0.7$  for 2019 and  $r = 0.71$  for 2020).

In both years, genotypes grown under drought showed lower seed and shoot  $\Delta^{13}C$  than those grown under irrigation, demonstrating the effectiveness of the rainout shelter to impose drought (Tables 4 and 5). In addition, in both years, seed  $\Delta^{13}C$  was significantly affected by genotypes under drought (Tables 4 and 5). In 2019, the highest seeds  $\Delta^{13}C$  value was found in PI 502120, followed by Tifrunner and PI 493938; meanwhile the lowest  $\Delta^{13}C$  value corresponded to two of the lowest yielding genotypes, CG7-A and PI 442768 (Table 4). Not all high-yielding genotypes showed high seed  $\Delta^{13}C$ . For instance, TifNV-High O/L, Line-8, and AU16-28 showed relatively low seeds  $\Delta^{13}C$ , which were the lowest within the drought-tolerant genotypes. Most drought-sensitive genotypes showed low seed  $\Delta^{13}C$ ; however, several drought-sensitive genotypes such as PI 493938 had high seed  $\Delta^{13}C$ . In 2020, the highest seeds  $\Delta^{13}C$  was 19.1‰ found in PI 493329, a drought-tolerant genotype, followed by Line-4 and AU-NPL 17; the lowest was found in PI 390428, a drought-sensitive genotype (Table 5). Of the high-yielding and drought-tolerant genotypes, line-8 showed the lowest  $\Delta^{13}C$  values, equaling PI 390428, a drought-sensitive genotype (Table 5). In 2019, shoot  $\Delta^{13}C$  did not show a significant effect on genotypes (Table 4), whereas in 2020 the genotype effect was significant (Table 5). In 2020, Line-4, a drought-tolerant genotype, showed the highest shoot  $\Delta^{13}C$ , and PI 325943, a drought-sensitive genotype, showed the lowest shoot  $\Delta^{13}C$  which were 20.5‰ and 18.9‰, respectively. Drought-tolerant genotypes Line-8 and TifNV High O/L showed lower shoot  $\Delta^{13}C$  values than Line-4 (Table 5). In 2019, seed and shoot  $\Delta^{13}C$  were positively correlated with yield, with seed  $\Delta^{13}C$  showing higher correlation ( $R^2 = 0.43$ ) with yield than shoot  $\Delta^{13}C$  ( $R^2 = 0.16$ ; Figure 2). In 2020, seed and shoot  $\Delta^{13}C$  showed significant positive correlation with yield ( $R^2 = 0.24$  and  $R^2 = 0.26$ , respectively), but the  $R^2$  was similar between the two organs and the  $R^2$  between seed  $\Delta^{13}C$  and yield was lower than in 2019 (Figure 2 and 3).

It is worth noting that in both years, drought-tolerant genotypes with medium photosynthetic rates, such as Line-8 and AU16-28, showed  $g_s$  and seed  $\Delta^{13}C$  values as low as drought-sensitive genotypes. However, these genotypes showed moderate to high photosynthesis and yields, with AU16-28 being the highest yielding in 2020, indicating that these genotypes may possess high WUE. Contrary to this, genotypes such as PI 502120, G06G, and AU-NPL17 showed high yields and high photosynthesis,  $g_s$ , and seed  $\Delta^{13}C$ , which may be associated with more efficient extraction and use of water from the soil.

### 3.3 | Relationship between physiological parameters

In both years, photosynthesis and  $g_s$  under drought were significantly and positively correlated with shoot biomass at any point in time, with the average of all the time points showing a stronger correlation (Figures 2 and 3). In 2019, seed  $\Delta^{13}C$  showed significant positive correlation with photosynthesis and  $g_s$  at all time points; meanwhile shoot  $\Delta^{13}C$  correlation with photosynthesis and  $g_s$  showed a positive correlation but much weaker than seed  $\Delta^{13}C$  (Figure 2). In 2020, seed  $\Delta^{13}C$  showed a positive correlation with photosynthesis and  $g_s$  at all points but was lower than in 2019; however, there was no correlation between shoot  $\Delta^{13}C$  and photosynthesis or  $g_s$  (Figure 3).

## 4 | DISCUSSION

This study consisted of irrigated and drought treatments. The mid-season drought treatment was successfully achieved by the utilization of the rainout shelters, causing mean yield losses of 57.5% and 35.2% in 2019 and 2020, respectively, which is similar to other peanut trials under true drought field conditions (Balota, 2020; Rucker et al., 1995). Some greenhouse studies indicated that mid-season drought, the type of drought that was implemented in this experiment, can cause a decrease in yield of approximately 35% (Carvalho et al., 2017; Junjittakarn et al., 2014). Previous field experiments have shown that there was a 53% yield loss under drought (Songsri et al., 2008a). Therefore, the observed physiological and yield reductions caused by the rainout shelters in this experiment can be considered normal and comparable to other peanut field experiments.

The lower yield reduction in 2020 (35.2%) in comparison with 2019 (57.5%) was probably due to more wet and cooler conditions (Figure 1). In 2020, the average maximum temperature was 1.2°C lower than in 2019 and had 110 mm more precipitation during the growing season. The fact that 2020 was a cooler and wetter year than 2019 likely explains why in this year the genotype differences in physiological parameters such as  $\Delta^{13}C$ , photosynthesis, and  $g_s$  under drought is significant but smaller than in 2019 (Tables 4 and 5). This smaller difference between genotypes has been shown before in common bean under irrigated conditions (Polania et al., 2016; Sanz-Saez et al., 2019) and is due to the fact that some genotypes with high plasticity only show significant differences when they are under extreme drought conditions (Tardieu et al., 2018).

Various peanut genotypes previously known by its drought tolerance and sensitivity when tested under different environmental and experimental conditions were tested in this study all under the same environment showing significant differences in yield under drought conditions in 2019 and 2020 (Tables 2 and 3). Among the drought-tolerant genotypes, PI 502120 and PI 493329 in 2019, and AU16-28 and G06G in 2020 were the highest yielding genotypes. Our results coincide with previous research that showed PI 493329 and G06G as drought tolerant (Balota, 2020; Otyama et al., 2019). On the other hand, genotypes PI 339960, PI 390428, and AP-3 (in 2020) showed the lowest yields under drought in both years (Tables 2 and 3), therefore demonstrating that these genotypes are drought sensitive. For example, genotype AP-3 has shown sensitivity to drought in previous research (Dang et al., 2012; Dang et al., 2013).

A majority of the drought-tolerant genotypes (PI 502120, PI 493329, Line 8, G06G, AU-NPL-17, and others) showed a high HI under drought (Tables 2 and 3). This, together with the high correlation between yield and HI under drought (Figure 2 and 3), demonstrate that this trait is very important for drought tolerance. The capacity of plants to remobilize their accumulated vegetative biomass into the pod has been recognized as one of the most important drought-tolerant traits in peanut (Vadez & Ratnakumar, 2016; Wright et al., 1991). Harvest index is a very important trait for drought tolerance because if a given genotype is able to tolerate drought and grow due to mechanisms such as an increased WUE but later is not able to remobilize that biomass to the pod, the yield and drought tolerance would be low. For this reason, it is important that breeders and physiologist select genotypes with different drought-tolerant mechanisms that always maintain high HI under drought.

Carbon isotope discrimination ( $\Delta^{13}\text{C}$ ) of seeds and shoots has been used in the past to estimate yield and WUE (Condon et al., 2002; Kaler et al., 2018; Wright et al., 1994; Zhou et al., 2016). In our study, seed  $\Delta^{13}\text{C}$  showed a stronger positive relationship with yield than shoot biomass (Figures 2 and 3), similar to what has been found in common bean (Polania et al., 2016; Sanz-Saez et al., 2019). This would indicate that seed  $\Delta^{13}\text{C}$  is a better parameter than shoot  $\Delta^{13}\text{C}$  to predict yield; however, studies in soybean have demonstrated that shoot and seed  $\Delta^{13}\text{C}$  can both be used to predict yield (Dutra et al., 2017; Kaler et al., 2018). The low correlation between shoot  $\Delta^{13}\text{C}$  and yield observed in our study (Figures 2 and 3) was likely due to the fact that the samples were taken at harvest and at that point some of the  $^{13}\text{C}$  fixed in the shoot may have already been transported to the reproductive organs (Kvien et al., 1986; Zhou et al., 2016).

In the literature,  $\Delta^{13}\text{C}$  has been also used to screen for “water user” and water saver genotypes for breeding purposes (Blum, 2009; Polania et al., 2016). In our study, we found that seed  $\Delta^{13}\text{C}$  showed a positive relationship with yield and

was able to differentiate between these two types of genotypes. With this technique, we were able to identify Line-8 and AU16-28 as water saver genotypes (have high WUE) because they showed lower  $\Delta^{13}\text{C}$  than other drought-tolerant genotypes, such as PI 502120, PI493329 and AU-NPL 17 (Tables 4 and 5), but with similar yields (Tables 2 and 3; Figure 5). Low  $\Delta^{13}\text{C}$  has been related with high WUE in previous research and used to select drought-tolerant genotypes in other crops such as wheat, common bean and peanut genotypes (Blum, 2009; Condon et al., 2002; Hubick et al., 1986; Passioura, 1977; Rao et al., 1993; Wright et al., 1988; Wright et al., 1994). In contrast, high  $\Delta^{13}\text{C}$  has been used to select for high EUW (Blum, 2009; Polania et al., 2016). Efficient use of water refers to the amount of water used in transpiration under limited soil water condition to produce high biomass and yield (Blum, 2009). Genotypes with high EUW, water spenders, are able to maintain high transpiration and therefore photosynthesis that results in higher biomass accumulation and yield (Blum, 2009; Polania et al., 2016). In our study, we found that genotypes PI 502120 and AU-NPL 17 showed high yields with high  $\Delta^{13}\text{C}$  (Tables 2–5; Figure 4) in both years, which would classify them as high EUW genotypes according to Polania et al. (2016).

The stable natural abundance  $\Delta^{13}\text{C}$  technique was used in this study to determine the integral WUE and EUW for the entire growing season in the whole plant following the methods in previous studies (Blum, 2009; Condon et al., 2002; Polania et al., 2016). Although this technique is useful, it is expensive, costing \$9.5 per sample, and very time consuming as a lot of labor is required to collect and prepare the samples for isotope analysis. In addition, this technique is not very sensitive to small changes in transpiration at some time points during the growing season due to changes in  $g_s$  (Nautiyal et al., 2012). For example, the separation between water spender and water saver genotypes was not very clear when only using the  $\Delta^{13}\text{C}$ . In both years, the water spender genotype, AU-NPL 17, did not show significant differences in seed  $\Delta^{13}\text{C}$  with water saver genotypes AU16-28 and Line-8 (Tables 4 and 5, Figure 4). To have a clearer separation between these two drought tolerance mechanisms, we considered the average photosynthesis and  $g_s$  values. In this case, AU-NPL 17 and PI 502120, water spender genotypes, always showed higher  $g_s$  and therefore used more water than water saver genotypes AU16-28 and Line-8 (Tables 4 and 5, Figure 5). For this reason, we performed an intense campaign of gas exchange measurements taken with three portable gas exchange analyzers (LI-COR6400) during the drought period to test if these measurements could be used to better detect genotypes with high WUE and EUW than with the  $\Delta^{13}\text{C}$ . In addition to the precision of this method in separating between water spender and water savers, we found that the correlation between  $g_s$  and photosynthesis with seed  $\Delta^{13}\text{C}$  was high (Figures 2 and 3), similar to what has been described in

previous studies (Nautiyal et al., 2012). In addition, the correlation of yield with  $g_s$  and photosynthesis when these values were averaged through the drought period was higher than the correlation between yield and seed  $\Delta^{13}C$  (Figures 2–5). This was surprising because in previous research, the correlation between yield and gas exchange measurements has been reported as weak (Dutra et al., 2017; Zheng et al., 2011). The high correlation observed in our study is likely the result of averaging the abundant and repeated measurements during the drought period. For these reasons, we suggest that gas exchange measurements can better differentiate between water saver and water user genotypes or even substitute for the  $\Delta^{13}C$  to differentiate between these two drought-tolerant mechanisms.

In common bean, it has been demonstrated that water user genotypes also have a high  $\Delta^{13}C$  and that it results in higher yields than water saver genotypes (Polania et al., 2016; Sanz-Saez et al., 2019). This advantage of the water spender over the water saver is achieved through a higher transpiration that results in higher photosynthesis and thus biomass accumulation and yield (Blum, 2009; Polania et al., 2016). However, our data shows that in the studied lines of peanut, having a high EUW (higher  $\Delta^{13}C$ ) does not result in higher yields. For example, in 2019, the highest yielding genotype was PI 502120, which also showed the highest  $\Delta^{13}C$  and  $g_s$  and, thus was assumed to be a water spender. However, in 2020, the highest yielding genotype, AU16-28, showed a low  $\Delta^{13}C$  and one of the lowest  $g_s$  of all the drought-tolerant genotypes. Therefore, in peanut under mid-season drought in the Southeastern United States, both mechanisms of drought tolerance appear to be equally capable of producing high yields. However, to increase the sustainability of the peanut agricultural systems, water saver genotypes should be prioritized to save more soil water. In addition, more research is needed on the phenotypic variability of water saving and water user genotypes in more diverse peanut populations and under different environments to elucidate its genetic regulation.

## 5 | CONCLUSIONS

Plants that exhibit drought tolerance can be classified as water spenders or water savers. The objective of this study was to measure mechanisms leading to water spender or water saver characteristics in a diverse group of peanut genotypes in 2019 and 2020. We demonstrated that a rainout shelter facility was able to implement mid-season drought comparable to those experienced under field conditions. Previously selected drought-tolerant genotypes showed higher yields than drought-sensitive ones in both years of the study. Using carbon isotope discrimination and gas exchange

measurements, we were able to detect two drought tolerance mechanisms. Genotypes PI 502120 and AU-NPL 17 were classified as water spender genotypes, as they showed high yield,  $\Delta^{13}C$ , photosynthesis and  $g_s$  under drought. This study is the first time that water spender peanut genotypes have been identified. On the other hand, genotypes Line-8 and AU16-28 were classified as water savers as they showed equally high yields but with low  $\Delta^{13}C$ ,  $g_s$ , and moderate photosynthesis. In this study, water spender genotypes did not show a yield advantage in comparison with water savers ones, so both types of genotypes could be recommended to increase drought tolerance in the southeastern United States. However, water savers should be prioritized as they could be more environmentally sustainable due to high yields and low water consumption. In this study, we also demonstrated that abundant gas exchange measurements during the drought period were able to better separate between water saver and water user genotypes than using the  $\Delta^{13}C$  technique alone.

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## AUTHOR CONTRIBUTIONS

Qiong Zhang: Data curation; Formal analysis; Investigation; Methodology; Visualization; Writing – original draft. Phat Dang: Conceptualization; Funding acquisition; Methodology; Project administration; Writing – review & editing. Charles Chen: Conceptualization; Funding acquisition; Investigation; Methodology; Project administration; Writing – review & editing. Yucheng Feng: Methodology; Writing – review & editing. William Batchelor: Supervision; Writing – review & editing. Marshall Lamb: Writing – review & editing. Alvaro Sanz-Saez: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Writing – original draft; Writing – review & editing.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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