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Using carbon isotope discrimination to assess genotypic differences in drought resistance of parental lines of common bean (*Phaseolus vulgaris* L.)

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Abbreviations: Carbon isotope discrimination ($\Delta^{13}C$), Drought sensitive (DS), Drought resistant (DR), Harvest index (HI), Leaf area index (LAI), Mid-pod fill (MPF), Pod harvest index (PHI), Shoot dry weight (Shoot DW), Stomatal conductance (g_s), Terminal drought stress (TDS), Well-watered (WW), Water uptake (WU), Water use efficiency (WUE), Yield drought index (YDI).

Key words: water use efficiency, carbon isotope discrimination, common bean, water stress, yield.

ABSTRACT

Accurate assessment of crop water uptake (WU) and water use efficiency (WUE) are not easy under field conditions. Carbon isotope discrimination ($\Delta^{13}C$) has been used as a surrogate of WUE to examine crop yield responses to drought and its relationship with WU and WUE. A two-year study was conducted to (i) characterize genotypic variation in $\Delta^{13}C$, grain yield, and other physiological parameters in common bean (*Phaseolus vulgaris* L.) parental lines, and (ii) examine the relationships between grain $\Delta^{13}C$, shoot $\Delta^{13}C$, and grain yield under well-watered and terminal drought stress conditions. All measured plant traits were strongly influenced by water availability, and genotypic differences in grain yield, shoot $\Delta^{13}C$, and grain $\Delta^{13}C$ were found in both watered and terminal drought stress environments. The parental lines were classified into two drought adaptation groups, drought resistant and drought sensitive, based on a yield drought index. High yields under drought conditions were related to (1) greater water uptake, as indicated by high $\Delta^{13}C$ in genotypes previously shown to have deeper roots (e.g. SEA 5 and BAT 477), and (2) increased water use efficiency, denoted by lower $\Delta^{13}C$ and greater pod harvest index (PHI) (e.g. SER 16). Coupling of $\Delta^{13}C$ measurements with measured yield and yield components analyses, such as PHI, provided an avenue to distinguish different physiological traits among drought resistant genotypes underlying adaptation to water deficit stress.

INTRODUCTION

Current food production and annual crop yield gains are insufficient to meet the United Nations' target to double crop yields in response to a projected human population growth from 7.6 to 9.7 billion by 2050 (Tilman et al., 2011: Valin et al., 2014; United Nations, 2017). Common bean (*Phaseolus vulgaris* L.) is the most important food legume in tropical regions of Latin America and Sub-Saharan Africa and is commonly cultivated by subsistence farmers (Beebe et al., 2013). Limited use of fertilizer, insecticides, and irrigation inputs by these subsistence farmers usually result in low common bean yields (Beebe, 2012). Drought, either intermittent or terminal, occurs in approximately 60% of the common bean production area, with potential yield losses up to 100% (Beebe et al., 2013; Rao, 2014). Therefore, selection and breeding of drought resistant common bean varieties is necessary to increase food security in marginal areas in which irrigation during the dry season is either not available or is cost prohibitive (Beebe et al., 2013; Polania et al., 2016a, b).

Passioura (1977) defined seed yield of a crop under water-limited environments as the product of three factors: water uptake (WU), water-use efficiency (WUE), and harvest index (HI). According to Condon et al. (2004), the selection of traits that increase any of these three factors under drought is paramount for breeding crops grown in water-limited conditions. Previous research on common bean has primarily focused on increasing WU by selecting varieties with deeper roots (Rao, 2014; Polania et al., 2017a,b), and increasing HI by breeding for greater biomass accumulation during the vegetative stages and more efficient C remobilization from vegetative tissues to seeds (Rosales-Serna et al., 2004; Klaedtke et al., 2012; Rosales et al., 2012; Assefa et al., 2013). However, improvement of common bean WUE has received limited attention and usually is not a primary target in breeding programs, likely because it is difficult to quantify under field conditions (Araus et al., 2002; Easlon et al., 2012).

In crop production, WUE can be defined at various scales. Agronomic WUE is defined as yield per unit of irrigation and/or precipitation (Passioura, 1997), and physiological WUE is defined as the above ground biomass divided by the amount of water transpired (Condon et al., 2004). Plants grown under water-limited conditions generally display greater physiological WUE because the reduction in net photosynthesis in response to reduced stomatal conductance (g_s) is less than the reduction in transpiration (Farquhar and Sharkey, 1982; Gilbert et al., 2011; Medrano et al., 2015). Indeed, increased WUE is often associated with smaller plants and lower yield potential as it usually is the result of reduced water use rather than enhanced C assimilation per unit of water (Munoz et al., 1998; Martin et al., 1999; Blum et al., 2005; Richards 2006). Thus, mechanisms that increase WUE by shifting the relationship between net photosynthesis and water loss in favor of C assimilation, such as increasing photosynthetic efficiency and reducing cuticular transpiration (Kerstiens et al., 1996), are of great interest when breeding cultivars for high yields under water-limited conditions.

Carbon isotope discrimination ($\Delta^{13}C$) signatures of shoot biomass or seed samples have been demonstrated to be negatively correlated with physiological WUE in several crop species, including common bean (Farquhar et al., 1989; Ehleringer et al., 1991; El-Sharkawy et al., 2007). White et al. (1990) found that some common bean cultivars with deep roots were able to access more water under drought conditions, and that reflected in higher $\Delta^{13}C$ signatures and lower WUE. In subsequent work, White et al. (1994a, b) examined the relationship between $\Delta^{13}C$ and yield in F2 and F3 populations from a nine-parent diallel without reciprocal crosses but did not find a consistent relationship and suggested that this may have been due to differences in root system and leaf characteristics. Interestingly, Polonia et al. (2016a, b) successfully used $\Delta^{13}C$ of shoot and seed tissues to predict common bean yield response under irrigated and rain-fed conditions. Like common bean, observations in other species also indicate complex relationships of $\Delta^{13}C$ and seed yield (Brito et al., 2014; Vadez et al., 2016). For instance, in wheat (*Triticum aestivum*), $\Delta^{13}C$ signature and yield were positively correlated under moderate drought conditions (Araus et al., 1998; Fischer et al., 1998; Merah et al., 2001), but negatively correlated under severe drought conditions (Del Pozo et al., 2016). Although relationships with yield can be inconsistent, $\Delta^{13}C$ signatures generally closely relate to g_s and WUE, and as such can provide valuable information about physiological mechanisms associated with drought resistance. In contrast to physiological measurements such as leaf-level photosynthesis and stomatal conductance, which reflect plant status at a particular moment in time (minutes), whole-plant and seed $\Delta^{13}C$ signatures have the advantage that they integrate photosynthesis and transpiration status of a plant over a long period of time (weeks, season), and can be readily determined for a large number of plants (Farquhar et al., 1989; Easlon et al., 2014).

Researchers at the International Center for Tropical Agriculture (CIAT) select and develop drought-resistant common bean varieties, with much of the selection having integrated grain yield and several morpho-physiological traits such as pod harvest index (PHI), g_s, deep rooting, and high vigor under drought (Beebe et al., 2013; Rao, 2014; Polania et al., 2016a, b). Selected drought-resistant germplasm was crossed with germplasm containing additional positive traits including seed composition and disease resistance, to develop improved cultivars and recombinant inbred line populations for genetic studies (Polania et al., 2016b; Diaz et al., 2018). However, the genotypic variation of $\Delta^{13}C$ and its relationship to yield under conditions differing in water availability have not been studied in the parental lines of these CIAT mapping populations. Thus, the main objectives of this study were to (i) characterize genotypic variation in $\Delta^{13}C$, yield and other physiological parameters including leaf area index (LAI), g_s, PHI, hundred seed weight

(100SW), under well-watered (WW) and terminal drought stress (TDS) conditions and (ii) determine the relationship between these traits in 14 parental lines grown under TDS conditions.

MATERIALS AND METHODS

Field site, experimental design, plant material, and crop management

Two field studies were conducted at the main experimental station of CIAT near Palmira, Colombia (3° 29’ N, 76° 21’ W) at an altitude of 965 m, during the dry seasons (June to September) of 2012 and 2013. The weather data during the two seasons were collected at the CIAT weather station near the field sites and are summarized in Table 1. The basic field characteristics were described previously by Beebe et al. (2008). Briefly, experiments were conducted on a Mollisol (fine-silty, mixed, isohyperthermic Aquic Hapludoll) with adequate nutrient supply, a pH of 7.7, and a water holding capacity of 100 mm of available water (assuming 1.0 m of effective root growth with -0.03 MPa and -1.5 MPa as upper and lower limits for soil matric potential).

Two levels of water supply were applied to impose well-watered (WW) and terminal drought stress (TDS) conditions. Well-watered and terminal drought stress treatments were conducted in separate areas of the same field with four replications of 14 common bean genotypes. The cultivars were selected following three criteria (1) significance in the CIAT MesoAmerican and Andean breeding programs, (2) diversity in genetic backgrounds, and (3) are parents of existing recombinant inbred line mapping populations (Table 2). Originally, some of these genotypes were selected for specific traits including drought resistance and drought sensitivity. Drought resistant genotypes were selected based on deep rooting (BAT 477, SEA 5, White et al., 1990; Singh et al., 1991), high HI under drought (SER 16, Polania et al., 2016a), high shoot vigor under drought (SXB05, Assefa et al., 2013), and drought and low fertility adaptation (BFS10, Beebe et al., 2008; Suarez-Salazar et al., 2018), and genotypes described as drought resistant by

CIAT due to their high yields under drought conditions (DAB 295, DAB 494, Polania et al., 2016a; Suarez-Salazar et al., 2018). Drought sensitive lines were selected for their commercial use and known sensitivity to drought (Assefa, 2013; Polania et al., 2016b, 2017; Diaz et al., 2018).

Land preparation to establish the field experiments was according to the standard practices to assure normal growth of the crop (Beebe et al., 2013). The TDS experiments were planted on 3 Aug. 2012 and 15 July 2013, and the WW experiments were planted on 10 Aug. 2012 and 18 July 2013. Each genotype was planted in four-row plots measuring 3.72 m in length and 2.4 m in width to achieve a stand density of 240,000 plants ha^{-1}.

Water availability was managed using furrow irrigation as follows: TDS experiments were irrigated three times (35 mm per irrigation) between planting and one week before flowering in both years. Irrigation was suspended after the third irrigation to induce terminal drought conditions. To ensure adequate soil moisture availability for vigorous growth, WW experiments were irrigated 5 times in 2012 and 6 times in 2013 with 35 mm of water per irrigation.

In both growing seasons, no fertilizer applications were made but fields were managed with herbicides to control weeds (Fomesafen, Fluazifop-p-butil, and Bentazon), with insecticides to control pests (Thiametoxam, Chlorpyrifos, Imidacloprid, Abamectin, Cyromazine, and Milbemectin), and with fungicides (Benomyl and Carboxin) to control fungal infections as needed. Since common beans have been planted in these fields for more than 30 years, no inoculations with *Rhizobium* were needed.

Physiological, yield, and harvest index measurements

Maximum aboveground biomass of common bean is generally achieved at mid-pod fill (MPF) development stage (Beebe et al., 2013), therefore all physiological measurements were performed at that stage. Stomatal conductance on one fully expanded leaf (3rd or 4th leaf from the shoot apex) was measured per plot using a leaf porometer (Decagon SC-1, Meter Environment, Inc. Pullman, WA, USA). Measurements were performed between 11:00 and 13:00 h on a clear sunny day. Shoot samples were also collected at MPF from a 0.5 m long section of one outside row per plot. Plants were counted and cut approximately 10 mm above the soil surface and were separated into leaves, stems, and pods. Leaf area was measured using a LI-3100C leaf area meter (LI-COR Biosciences, Lincoln, Nebraska, USA) and used to calculate leaf area index (LAI). After drying in a forced-air oven at 60°C, sample weights were determined.

At physiological maturity, and prior to harvest for grain yield, plants from a 0.5 m long section of one of the two center rows were cut and used to determine the weight of 100 seeds (100SW), and PHI according to Beebe et al. (2013). Grain yield per plot was measured from the two center rows after trimming plants within a 0.3 m border off each end of each row and then hand shears were used to cut the plants at the soil surface. Yields per hectare were calculated and are reported on a 0% grain moisture basis. The yield drought index (YDI) was calculated for each plot as:

$$Yield\ Drought\ Index = \frac{Yield_{WW} - Yield_{TD}}{Yield_{TD}}$$

where, $Yield_{WW}$ was the yield of each genotype and plot in WW conditions, and $Yield_{TD}$ was the yield of each genotype and plot in TDS conditions.

Determination of carbon isotope discrimination ($\Delta^{13}C$)

The dry biomass from the samples collected at MPF and the seed obtained at maturity were ground to pass a 1 mm screen, weighed into tin capsules, and shipped to the UC-Davis Stable Isotopes Facility (Davis, California, USA) for C isotope analysis. Samples were analyzed using an isotope ratio mass spectrometer (IsoPrime, Elementar France, Villeurbanne) coupled to an elemental analyser (EA3000, EuroVector, Milan, Italy). The $^{13}C/^{12}C$ ratio (R) in plant material was first calculated in δ notation ($\delta^{13}C$, carbon isotope composition) with respect to Vienna Pee Dee Belemnite calcium carbonate (V-PDB), with an analytical precision of 0.1‰, and later transformed to apparent C isotope discrimination ($\Delta^{13}C$, ‰).

$$\delta^{13}C = \left(\frac{R_{sample}}{R_{standard}}\right) - 1$$

$\delta^{13}C$ accuracy was monitored using international secondary standards of known $^{13}C/^{12}C$ ratios (IAEA-CH7 polyethylene foil, IAEA-CH6 sucrose and USGS-40 glutamic acid, IAEA, Austria), and internal controls of known $^{13}C/^{12}C$ (Bovine liver, peach leaves, nylon 5). The apparent C isotope discrimination ($\Delta^{13}C$, ‰) was calculated as:

$$\Delta^{13}C = \frac{\delta^{13}C_{atm} - \delta^{13}C_{sample}}{\delta^{13}C_{sample} + 1}$$

Where $\delta^{13}C_{atm}$ is the carbon isotope composition of atmospheric CO_2 (-8 ‰; Farquhar et al., 1989) and $\delta^{13}C_{sample}$ is the carbon isotope composition of the plant sample (above-ground biomass or seed).

Statistical analysis

To determine if genotypes differed in the measured traits under different water availability, mixed model analysis of variance (ANOVA) using PROC MIXED in SAS 9.4 (SAS Institute, Cary NC, USA) was performed with year, genotype, and water availability (later called environment) modelled as fixed effects, and replication as a random effect. The year effect was significant ($p \leq 0.001$) for all parameters with the exception of LAI ($p = 0.343$) and PHI ($p = 0.166$) and showed interaction with the other fixed effects, therefore all data were reanalyzed separately by year, using a mixed model ANOVA with genotype and environment as fixed effects, and replication as random effect. Because the TDS and WW treatments were conducted in the same field but side-by-side, they were considered separate water environments (or location) in the analysis, and the environment by genotype interaction effect was used to evaluate the stability of the genotypes with respect to each trait. A separate mixed model ANOVA was conducted for the yield drought index, with genotype as fixed effect and replicate as random effect. To test for significant differences between genotypes, paired linear contrasts were performed on the least square means.

Based on previous studies, all genotypes included in this study were then classified as drought resistant or drought sensitive (see Table 2 for the classification). Thus, after identifying genotypic differences for each measured parameter, the relationships between the classification of a genotype as drought resistant or drought sensitive and the parameters measured in this study were examined. To this end, data were analyzed using a mixed model ANOVA with environment and "drought adaptation" category as fixed effects and replicate as a random effect.

Correlations and linear dependencies among yield, DW, shoot $\Delta^{13}C$, grain $\Delta^{13}C$, g_s, LAI, PHI, and 100SW were tested using PROC CORR (SAS 9.4).

RESULTS

Environment and genotype effects

The TDS environments received almost 27% and 36% less water than the WW environments in 2012 and 2013, respectively, with similar temperatures and total radiation for the two growing seasons (Table 1). The difference in the amount of water received in the TDS and WW environments resulted in significant differences between the environments for all the measured parameters in both years (Table 3). In 2012, the genotype effect was significant for all parameters except for shoot DW and g_s. In addition, only PHI and 100SW showed significant genotype by environment interactions. In 2013, genotype was significant for all but three parameters: shoot DW, LAI, and g_s (Table 3), and the genotype by environment interaction was significant for shoot $\Delta^{13}C$, LAI, PHI and 100SW (Table 3).

Mean grain yields in 2013 were greater than in 2012 in both WW and TDS environments, and TDS significantly reduced yields of all genotypes in both years (Fig. 1). On average across genotypes, yields in TDS were only 48.4% and 42.8% of those observed in WW environments in 2012 and 2013, respectively. Yield differences among genotypes were more pronounced in the TDS than WW environments (Fig. 1). The lowest-yielding genotypes in the WW environments produced 57.3% and 51.9% of the highest-yielding genotypes in 2012 and 2013, respectively. In the TDS environments, the lowest-yielding genotypes only produced 25.1% and 31.3% of the highest-yielding genotypes in 2012 and 2013, respectively. These yield responses resulted in significant genotype effects ($P < 0.1$ in 2012 and $P < 0.05$ in 2013) when calculating the YDI (Table 2). The penalty to grain yield in the TDS environment compared to WW was greater on average than in shoot DW and LAI, but each parameter was lower in TDS than in WW environments in both years (Table 3; Fig. 1). Average shoot DWs across all genotypes in TDS were 69.8% and 64.3% of those under WW conditions in 2012 and 2013, respectively. Average LAIs in TDS were only 61.6% (2012) and 60.5% (2013) of those in WW.

Environment and genotype effects were highly significant for both shoot $\Delta^{13}C$ and grain $\Delta^{13}C$ in both years (Table 3; Fig. 2). Genotype by environment interactions were not significant in 2012, and in 2013 the interaction effect was significant for shoot $\Delta^{13}C$ only. On average, shoot $\Delta^{13}C$ in the TDS environment was reduced by 6.4% (2012) and 8.8% (2013) in comparison with the WW environments. Similarly, grain $\Delta^{13}C$ in the TDS environments was reduced by 8.1% (2012) and 9.6% (2013) compared to the values determined for the WW environments. Correlation analyses between shoot $\Delta^{13}C$ and grain $\Delta^{13}C$ from both TDS and WW environments showed significant positive relationships in 2012 ($r = 0.70$, $p \leq 0.001$) and 2013 ($r = 0.61$, $p \leq 0.001$). When examined by environment, correlations between shoot $\Delta^{13}C$ and grain $\Delta^{13}C$ were significant in both environments in 2012, but only in TDS in 2013 (Supp. Table 1). In both years and environments, average shoot $\Delta^{13}C$ was greater than average grain $\Delta^{13}C$ (Fig. 2), but the environment did not appear to influence the relationship between shoot $\Delta^{13}C$ and grain $\Delta^{13}C$ in that the shoot-grain $\Delta^{13}C$ ratio was 1.10 and 1.12 in TDS environments and 1.08 and 1.10 in WW environments in 2012 and 2013, respectively. .

Highly significant environment, genotype, and genotype by environment interactions were observed for PHI and 100SW in both years (Tables 3 and 4). Both PHI and 100SW averages across genotypes were greater in WW than in TDS environments in 2012 and 2013. The strong genotype by environment interaction for PHI is illustrated by the 11.6% reduction in PHI between the WW to TDS environments in the least stable genotype (CAL 143) compared to 1.5% reduction in the most stable genotype (SEA 5). Similarly, the 100SW of the most responsive genotype (CAL 96) was 22.9% greater in WW than in TDS whereas the 100SW of the most stable genotype (G21212) differed by only 6.5% between the two environments. Interestingly, despite the highly significant genotype by environment interactions observed for both PHI and 100SW, this interaction effect was not significant for grain yield.

Genotypes included in this study were classified as either drought resistant (DR) or drought sensitive (DS) based on previous research (Table 2). When ANOVA was conducted by the drought adaptation grouping, the environment effect was significant for all parameters in both years, and the drought adaptation was significant for all tested parameters except for LAI and g_s (in both years) and shoot $\Delta^{13}C$ in 2012 (Table 3). However, a significant interaction between drought adaptation and environment was observed for LAI in 2013, but no significant interactions were observed for the other parameters (Table 3). Drought adaptation group significantly affected the YDI in both years, with DS genotypes exhibiting greater YDIs than DR genotypes (Table 3). Drought resistant genotypes showed greater grain yield, grain Δ^{13}C, and PHI in both years and in both WW and TDS environments compared to the DS genotypes (Tables 3 and 4; Figs. 1 and 2). Additionally, in the TDS environment, DR genotypes displayed greater shoot DW than DS genotypes in both years. For all other traits (shoot Δ^{13}C, LAI, g_s, 100SW), the differences between the DR and DS groups were either not significant or only significant in one of the two years.

Yield relationship with studied traits

Grain yield was positively correlated with all measured parameters in both years, when examined over both TDS and WW environments (Table 5). However, when WW and TDS environments were assessed separately, only grain Δ^{13}C and PHI were significantly correlated to grain yield in both environments and years. For all other traits, correlations were either not significant, not significant in one of the two environments, or only significant in one of the two years. For instance, when separated by environment, shoot Δ^{13}C was only positively correlated to grain yield in the TDS environment in 2013 (Table 5).

To examine which genotypes were driving the yield response in the TDS environments, and the relationship with Δ^{13}C signature, the mean yield of each genotype in TDS was plotted against grain Δ^{13}C and shoot Δ^{13}C by year (Fig. 3). Grain yield was positively correlated with shoot Δ^{13}C in 2013 but was not correlated with shoot Δ^{13}C in 2012 when no distinction was made for drought adaptation group. However, when the correlations were examined by drought adaptation group, grain yield was correlated with shoot Δ^{13}C for DR but not DS genotypes in 2012. Genotype SEA 5 exhibited the highest shoot Δ^{13}C and high grain yield in 2013, and SEA 5 and

G21212 had high shoot Δ^{13}C and grain yield in 2012 (Fig. 3). Grain yield was positively correlated with grain Δ^{13}C when analyzed for all genotypes; and when the genotypes were pooled by drought adaptation, the grain yield was positively correlated with grain Δ^{13}C for the DR genotypes in both years (Fig. 3). Again, genotypes SEA 5 and G21212 exhibited high grain yield and high grain Δ^{13}C in 2012 (Fig 3). In contrast, CAL 143 was consistently low in grain yield and in grain Δ^{13}C and shoot Δ^{13}C.

To explore the utility of carbon isotope discrimination as a tool to predict yield and yield losses under drought conditions, the relationship between YDI and shoot Δ^{13}C and grain Δ^{13}C in TDS conditions was plotted in Fig. 4. When no consideration was given to the drought adaptation group of the genotypes, YDI was consistently negatively correlated with shoot Δ^{13}C and grain Δ^{13}C in both years, indicating that the genotypes with higher shoot Δ^{13}C and grain Δ^{13}C under drought maintained greater yields. When the genotypes were separated by drought adaptation group, the DR genotypes also showed negative correlation between YDI and shoot Δ^{13}C, but no significant relationship was found for those in the DS group (Fig. 4). SEA 5 was the genotype with the lowest YDI and highest shoot Δ^{13}C in both years (Fig. 4), but exhibited the highest grain Δ^{13}C only in 2012. In 2013, BAT477 and SEA 5 had the lowest YDI and similar grain Δ^{13}C, which was lower than grain Δ^{13}C of other genotypes (DAB 295, SXB 405, BAT 881) with higher YDI (Table 2; Fig. 4).

DISCUSSION

Impact of water availability on yield, and physiological and morphological traits

Genotypic differences in Δ^{13}C of shoot DW harvested at MPF as well as in grain Δ^{13}C were found in both years and in WW and TDS environments (Table 3, Fig. 2). Plants grown under water-limited conditions exhibited lower Δ^{13}C values than plants grown under irrigated conditions, which is consistent with lower WUE of well-watered plants compared to drought-stressed plants observed in previous common bean experiments (White et al., 1994a,b; Beebe et al., 2013, Polania et al., 2016a). The absence of significant genotype by environment interactions for grain $\Delta^{13}C$ in either of the two years indicates that the genotypes examined in this study responded similarly under WW and TDS conditions. Interestingly, there was a genotype by environment interaction for shoot $\Delta^{13}C$ in 2013. It is unclear why this did not translate to a significant genotype by environment interaction for grain $\Delta^{13}C$, but the differences in shoot $\Delta^{13}C$ between cultivars may have been reduced by increasing stress severity after shoot biomass sampling. Additionally, C isotope fractionation as a result of remobilization from vegetative to reproductive tissues may also play a role (Zhou et al., 2014; 2015). Nonetheless, shoot $\Delta^{13}C$ and grain $\Delta^{13}C$ revealed significant positive correlations when analyzed across WW and TDS environments in each of the two years ($r = 0.70$ and $r = 0.61$). Interestingly, the correlation coefficients for 2012 and 2013 were comparable to those reported by Kaler et al. (2018) for soybean (*Glycine max*) in which shoot $\Delta^{13}C$ and grain $\Delta^{13}C$ from rainfed experiments conducted at three locations exhibited a strong relationship ($r = 0.69$). Unlike soybean (Kaler et al., 2018), the genotypic variation in the present study was greater for shoot $\Delta^{13}C$ ($F = 8.47$) than grain $\Delta^{13}C$ ($F = 6.05$). However, as in Kaler et al. (2018), the effect of environment was stronger for shoot $\Delta^{13}C$ ($F = 361.5$) than for grain $\Delta^{13}C$ ($F = 103.9$).

In environments with limited precipitation, high rates of transpiration can rapidly reduce soil water content and result in stomatal closure, thus leading to lower C assimilation and biomass accumulation (Blum et al., 2005; 2009). In the present study, a single stomatal conductance measurement at MPF revealed lower g_s in TDS than in WW environments (Table 3), but no differences in g_s were observed among genotypes in either of the two years. Previously, Polania et al. (2016a) found genotypic differences among a larger collection of common bean cultivars under terminal drought but not under irrigated conditions. Given the importance of timing for g_s measurements and observed genotype differences in shoot $\Delta^{13}C$, it is likely that additional g_s measurements could have revealed differences among genotypes in the present study.

Shoot DW was quantified at MPF and, as expected, was reduced as a result of termination of irrigation shortly before flowering in the TDS treatments in both years (Table 3, Fig. 2). In this study, shoot DW was reduced by 33% in TDS compared to WW environments when averaged across genotypes and years. This is comparable to the 31% and 36% impact of TDS previously reported by Polania et al. (2016a) and Polania et al. (2017a) respectively. As observed for g_s, genotypic differences in shoot DW were not consistently observed. However, the DS genotypes accumulated less (17%) shoot DW than the DR genotypes in the TDS environments, but the two genotype groups were similar in the WW environments (Fig. 2). Accumulation of greater shoot biomass in water-limited environments has been related with higher yields, probably as a result of larger pools of C for translocation to the pods and seeds, and/or because of enhanced ability of the genotypes to access more water with deeper roots (Asefa et al., 2013; Rao 2014; Polania et al., 2017a,b).

Leaf expansion is very sensitive to water deficit stress (Davies and Zhang 1991; Pantin et al., 2011). Reduced water availability has been linked with smaller leaf area and LAI in common bean (Kalaydjieva et al., 2015). However, lines that were able to maintain a greater LAI under drought conditions as a result of deeper roots exhibited greater biomass accumulation and yields (Beebe et al., 2014; Rao, 2014; Polania et al., 2017). The sensitivity of leaf expansion was clear by the 1.7-fold (average across both years) larger LAI in the WW than in the TDS environments. Although genotype by environment interactions were not observed in 2012, the significant interaction effect in 2013 suggests that different genotypes may differ in sensitivity of leaf expansion to reduced water availability, or that variations in access to soil water as a result of differences in rooting depth resulted in distinct rates of water stress imposition among genotypes and thus leaf expansion (Beebe et al., 2014; Rao, 2014; Polania et al., 2017). For instance, BAT 477, which is known to have deep roots, was able to maintain its LAI under TDS in 2013. This was in stark contrast with other genotypes such as BAT 881, CAL 143, and DOR 364, which had LAIs in the TDS environment that were only about 39% of those in the WW environment. Similar to BAT 477, G21212 and SEA 5 were among the genotypes with high LAI under TDS, which was probably related to greater soil exploration for water and nutrients associate with deeper roots, as previously reported for these genotypes (White et al., 1990; Polania et al., 2009; Beebe et al., 2013; Polania et al., 2017b; Rao et al., 2017). However, since rooting depth was not measured in the present study, it is possible that mechanisms other than rooting depth (e.g. greater C remobilization) also could have played a role..

As expected, grain yields were significantly lower in TDS compared with WW environments (Fig. 1, Table 3). Although genotype by environment interactions were not significant in either year, previous studies provide support for the greater average yield of DR genotypes (51% of WW) than those classified as DS (36% of WW) under TDS in this study (Fig. 1, See Table 2 for references regarding drought resistant cultivars). Indeed, grain yields of DR genotypes were 1.7-fold those of some DS genotypes in the TDS environment. Thus, these results confirm previous reports of significant genotypic variation in drought resistance among common bean genotypes.

Relationships between yield and physiological and agronomic traits

Shoot DW has been related to grain yield in several crop species, including wheat (Araus et al., 2002; Zhou et al., 2014), soybean (Koester et al., 2014), and common bean (White et al., 1990; 1994a). Aboveground biomass usually decreases under drought stress, but genotypes showing higher DW accumulation under TDS normally exhibit greater grain yields because the nutrients accumulated in the biomass prior to the stress period can be remobilized to the seed (Rosales-Serna et al., 2004; Rao et al., 2013). In the present study, there was no genotypic variation in shoot DW accumulation at MPF, when the genotypes were considered individually (Fig. 1; Table 3). However, analysis based on the DS and DR groups, showed that resistant cultivars accumulated more biomass than the sensitive cultivars under drought conditions (Fig. 1; Table 3). This difference between groups may contribute to the positive relationship between shoot DW and grain yield under drought stress conditions even though differences between genotypes were not significant.

Previous common bean studies reported mixed results, varying from no relation between grain yield and shoot DW with Δ^{13}C (White et al., 1990; 1994a, b) to positive relationships only in rainfed conditions (Polania et al., 2016a). In the present study, positive relationships between shoot Δ^{13}C and grain yield and between grain Δ^{13}C and grain yield were found when examined across TDS and WW environments (Table 5). However, when the relationships between Δ^{13}C and grain yield were examined separately by environment, only the grain Δ^{13}C showed a positive relationship with grain yield under both water regimes (Fig. 3). These differences in the relationship between Δ^{13}C and grain yield depending on the tissue analyzed and the water environment have been observed before in common bean and wheat (Zhou et al., 2014; Del Pozo et al., 2016; Polania et al., 2016a). Given that shoot samples were collected earlier in the season in comparison with grain samples and with respect to the terminal drought stress imposition, the closer relationship of grain Δ^{13}C to grain yield was not surprising, and consistent with the Δ^{13}C signature integrating the plant response to environmental conditions over different time periods

(Farquhar et al., 1998; Condon et al., 2004). Indeed, at the time of shoot sampling (mid-pod fill), the plants had not experienced the full extent of the terminal drought stress environment, as indicated by the greater relative impact of the terminal drought stress on grain yield compared to shoot DW at MPF (Fig. 1). In addition, the lower $\Delta^{13}C$ value in grain in comparison with shoot tissue has been related with partitioning of C from the shoot to the grain (Polania et al., 2016a). Thus, grain $\Delta^{13}C$ may be better suited for prediction of yield under drought than shoot $\Delta^{13}C$ (Polania et al., 2016a). Although the relationship of shoot $\Delta^{13}C$ with grain yield was less robust than that of grain $\Delta^{13}C$, leaf or shoot $\Delta^{13}C$ is more directly related to physiological traits associated with WUE, and, as such, can provide important information about physiological mechanisms underlying plant responses to differential water availability (Farquhar et al., 1989; Condon et al., 2004).

Flag leaf and grain $\Delta^{13}C$ signature has been used as a selection criterion for WUE in wheat (Condon et al., 2004; Richards, 2006), and also as an indirect indicator of the effective use of water (Araus et al., 2002; 2008; Blum, 2009; Blum, 2015). Blum (2009), suggested that lines with high effective use of water (EUW) as compared to those with low EUW have greater access to water through deeper roots or other mechanisms that allow a more favorable plant water status and thus can fix more C through photosynthesis and exhibit higher $\Delta^{13}C$ signatures. Accordingly, plant varieties may be classified into anisohydric (water spenders) and isohydric (water savers) types (Blum, 2015). The genotypes which had greater grain yield under terminal drought stress in this study were classified into water spenders (high grain yield and high $\Delta^{13}C$) and water savers (high grain yield and low $\Delta^{13}C$), depending on shoot $\Delta^{13}C$ and grain $\Delta^{13}C$ and the corresponding relationships to grain yield (Fig. 3). When yield was compared with grain $\Delta^{13}C$, only SEA 5 and BAT 477 were consistently classified as water spenders, and SER 16 as water saver. The classification of SEA 5 and BAT 477 as water spenders is consistent with previous studies that identified them as deep rooting genotypes which can access water deeper in the soil profile (White et al., 1990; Rao, 2014; Polania et al., 2017b). On the other hand, the water saver genotype, SER 16, likely was able to produce high yields under water-limited conditions due to its capacity to remobilize previously-fixed photosynthates to seed (Polania et al. 2016a).

Shoot Δ^{13}C and grain Δ^{13}C also have been used to predict yield losses under drought conditions by studying relationships between Δ^{13}C and different drought indices (Ehleringer et al., 1991, Polania et al., 2016a). In the present study, YDI was negatively correlated with shoot Δ^{13}C which indicates that genotypes that were better able to maintain yields under drought had greater Δ^{13}C, again, likely because of greater access to water due to deeper roots (Fig. 4).

These results suggest that CIAT common bean parental lines, such as SEA 5 and BAT 477, have been selected indirectly for their higher use of water and consequently are able to maintain higher C assimilation and grain yield under terminal drought. On the other hand, some genotypes, such as SER16, had low shoot and grain Δ^{13}C, indicative of high WUE.

CONCLUSIONS

The common bean genotypes studied in this experiment differed in grain yield, shoot Δ^{13}C, grain Δ^{13}C, and other physiological parameters such as PHI, DW, and 100SW when grown under terminal drought conditions. Terminal drought decreased shoot Δ^{13}C, grain Δ^{13}C, and grain yield in all lines. Parental lines previously identified by CIAT with drought resistant traits, averaged 1.7-fold greater grain yields compared to parental lines grouped as drought sensitive. Among the DR lines, SEA 5 had high yields and high shoot Δ^{13}C and grain Δ^{13}C under terminal drought, which may indicate higher efficient uptake of water compared to other genotypes. On the other hand, high yields of SER 16 were associated with high PHI, and low shoot $\Delta^{13}C$ and grain $\Delta^{13}C$, suggesting high WUE and C remobilization capacity. More research is needed to dissect specific physiological mechanisms underlying the common bean genotypic differences in shoot $\Delta^{13}C$, grain $\Delta^{13}C$, and yield relating to WUE and efficient uptake of water, including assessment of gas exchange characteristics, rooting depth, and hydraulic conductivity.

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Table 1. Temperatures, water input (irrigation + rain), evaporation, and total radiation, from planting to crop maturity in 2012 and 2013.

Year	**Average daily Maximum Temperature**	**Average daily Minimum temperature**	**Water received by terminal drought plots**	**Water received by well-watered plots**	**Total Evaporation**	**Total Radiation**
	---------------- °C	----------------	------------------------	mm ------------------------		MJ m^{-2}
2012	31.0 ± 1.3	19.0 ± 1.2	185	255	373.3	1185.3
2013	29.9 ± 2.0	19.1 ± 1.0	183.1	288.1	333.2	1141.3

Crop Sci. Accepted Paper, posted 06/12/2019. doi:10.2135/cropsci2019.02.0085

Table 2. Yield drought index (YDI) and characteristics of selection for 14 common bean genotypes grown in 2012 and 2013 at Palmira (Colombia). Genotypes were grouped as drought resistant and drought sensitive based on previous studies. Genotype effects and drought adaptation group effects from the respective ANOVA are indicated at the bottom of the table.

Drought adaptation grouping	Genotypes	2012 YDI	2013 YDI	Characteristics of selection of each cultivar and references
Drought Resistant	BAT 477	0.479 abc	0.194 c	Deep root (White et al., 1990)
	BFS 10	0.508 abc	0.497 abc	Low fertility and drought adapted (Suarez-Salazar et al., 2018)
	DAB 295	0.588 ab	0.615 ab	Drought adapted
	DAB 494	0.418 bc	0.638 ab	Drought adapted
	G 21212	0.296 c	0.412bc	Low fertility adapted (Beebe et al., 2008)
	SEA 5	0.254 c	0.226 c	Deep root (Beebe et al., 2014)
	SER 16	0.440 bc	0.569 ab	High HI (Polania et al., 2016a)
	SXB 405	0.465 bc	0.446 abc	Drought adapted (Assefa et al., 2013)
	DR Mean	0.431 B	0.449 B	
Drought Sensitive	BAT 881	0.742 a	0.769 a	Commercial line (Diaz et al., 2018)
	CAL 96	0.505 abc	0.756 a	Commercial line (Polania et al., 2016b)
	CAL 143	0.587 ab	0.710 ab	Commercial line (Polania et al., 2016b)
	DOR 364	0.674 ab	0.645 ab	Commercial line (Polania et al., 2016b)
	ICA BUNSI	0.441 bc	0.591 ab	Commercial line (Assefa et al., 2013)
	MD 23-24	0.583 ab	0.408 bc	Commercial line (Polania et al., 2017a)
	DS mean	0.589 A	0.647 A	
ANOVA effects by		(p-values)		
Genotype (G)		0.078	0.022	
Drought Adaptation (DA)		0.005	0.006	

Means followed by lower case letters represent the LSD test results for genotypic differences within each environment. Capital letters represent the LSD test results for differences between drought resistant and drought sensitive lines. Means followed by the same letter are not significantly different from each other (P =0.05).

Table 3. Analysis of variance (*p-value*) for grain yield, grain $\Delta^{13}C$, shoot dry weight at mid pod filling (MPF), and shoot $\Delta^{13}C$ at MPF measured in 2012 and 2013 for 14 common bean genotypes grown under well-watered or terminal drought conditions. The upper part of the table shows ANOVA results considering the 14 common bean genotypes and water environments as independent variables. The lower part of the table shows ANOVA results considering the drought adaptation classification of each genotype as drought resistant or drought sensitive (Table 2).

Year	**Effect**	**Grain Yield**	**Grain $\Delta^{13}C$**	**Shoot DW**	**Shoot $\Delta^{13}C$**
ANOVA by genotypes					
2012	Environment (E)	<0.0001	<0.0001	0.137	<0.0001
	Genotype (G)	<0.0001	<0.0001	0.413	<0.0001
	WE x G	0.347	0.751	0.612	0.159
2013	Environment (E)	<0.0001	<0.0001	<0.0001	<0.0001
	Genotype (G)	0.0004	<0.0001	0.186	0.0001
	E x G	0.076	0.09	0.318	0.006
ANOVA by drought adaptation grouping					
2012	Environment (E)	<0.0001	<0.0001	0.0003	<0.0001
	Drought Adaptation (DA)	<0.0001	<0.0001	0.0325	0.3577
	E x DA	0.877	0.832	0.819	0.688
2013	Water Environment (E)	<0.0001	<0.0001	<0.0001	<0.0001
	Drought Adaptation (DA)	<0.0001	0.014	0.044	0.0071
	E x DA	0.398	0.149	0.295	0.438

Crop Sci. Accepted Paper, posted 06/12/2019. doi:10.2135/cropsci2019.02.0085

Table 4. Leaf area index (LAI), stomatal conductance (g_s, µmol H_2O m^{-2} s^{-1}), pod harvest index (PHI), and hundred seed weight (100 SW, g of hundred seed) mean values of 14 common bean genotypes grown under well-watered (WW) and terminal drought stress (TDS) conditions in 2012 and 2013. Analysis of variance (*p-value*) for the same parameters measured in 2012, and 2013. The upper part of the table shows ANOVA results considering the 14 common bean genotypes and water environments as independent variables. The lower part of the table shows ANOVA results considering the drought adaptation classification of each genotype as drought resistant or drought sensitive (Table 2).

Drought Adaptation grouping	Lines	2012								2013							
		LAI†		g_s†		PHI‡		100 SW‡		LAI†		g_s†		PHI‡		100 SW‡	
		WW	TDS	WW	TDS	WW	TDS	WW	TDS	WW	TDS	WW	TDS	WW	TDS	WW	TDS
Drought Resistant	BAT 477	4.35 ab	2.38 abcd	425.40	368.70	74.7 cd	70.3 abcd	24.4 fe	21.4 f	2.86 d	2.95 a	461.88	73.85	72.3 de	72.7 b	19.2 hi	17.7 de
	BFS 10	3.2 cd	1.87 cdef	446.20	292.65	79.4 ab	76.3 a	29.1 d	25.9 cde	3.77 abcd	2.26 abc	446.97	92.60	78.9 a	79.2 a	26.7 de	22.7 bcd
	DAB 295	3.36 cd	1.89 cdef	453.08	384.28	75.7 bcd	72.2 abcd	51.1 b	39.5 a	3.44 bcd	2.71 ab	360.02	55.76	75.3 abcd	71.2 bc	49.7 ab	39 a
	DAB 494	2.21 fe	1.81 ef	455.90	390.50	78.4 abc	72.4 abcd	49.2 b	40.9 a	2.9 d	1.62 c	375.50	69.50	76.6 abc	71.3 bc	47.7 b	34.7 a
	G 21212	4.48 a	2.62 ab	433.60	401.73	79.2 ab	74.6 ab	30.2 d	27.2 cd	2.66 d	2.66 ab	348.22	57.45	77.1 abc	74.1 ab	23.5 fg	22.7 bcd
	SEA 5	3.94 abc	2.88 a	582.43	318.45	76.9 bcd	75.2 ab	28.8 d	25.8 cde	3.5 bcd	2.04 abc	359.30	111.48	76.3 abc	75.7 ab	27.7 d	24.2 bc
	SER 16	3.13 cde	1.82 ef	499.58	401.15	82 a	76.6 a	28.1 de	23.3 def	3.84 abcd	1.87 bc	425.20	74.73	79.1 a	76.4 ab	24.7 ef	19 cde
	SXB 405	3.16 cde	2.36 abcde	343.88	431.38	77.7 bcd	68.4 bcde	29.7 d	28.3 c	3.31 bcd	1.9 bc	376.78	49.63	76.6 abc	76.3 ab	26 de	21.2 bcd
	DR Mean	3.48 A	2.21 A	455 A	373.6 A	77.7 A	68.4 A	33.8 A	29.4 A	3.29 B	2.43 A	394.2 A	73.1 A	76.5 A	74.6 A	30.6 A	25.1 A
Drought Sensitive	BAT 881	3.35 cd	1.81 ef	522.68	487.73	74.3 de	65.4 de	20.5 gh	16.2 g	4.09 abc	1.66 c	525.12	104.75	74.1 cde	66.1 cd	17.5 ij	13.7 e
	CAL 96	2.94 de	1.81 ef	501.28	370.23	78.4 abc	68.2 bcde	56.7 a	43.6 a	2.94 cd	1.88 bc	461.65	68.28	76.3 abc	70.3 bc	51.7 a	40 a
	CAL 143	3.18 cde	2.4 abc	473.48	439.83	70.6 e	60.6 e	41.6 c	34.4 b	4.79 a	1.74 bc	259.65	131.10	70.6 e	64.2 d	37.2 c	27.2 b
	DOR 364	3.42 bcd	1.84 def	410.03	388.45	76.5 bcd	66.1 cde	22.4 fg	21.3 f	4.35 abc	1.78 bc	377.42	91.10	74.8 bcd	73.4 ab	21.5 gh	16.7 de
	ICA BUNSI	2.98 cde	1.69 f	410.40	499.58	76.6 bcd	68.3 bcde	18.4 h	14.8 g	3.38 bcd	2.32 abc	370.77	115.45	74.4 cd	72.3 bc	15.7 i	14.5 e
	MD 23-24	3.92 abc	2.15 bcdef	434.93	339.58	78.6 ab	74.3 abc	23.9 fg	21.9 ef	3.71 abcd	2.61 abc	433.23	132.70	78.6 ab	77.3 ab	21 h	18 de
	DS mean	3.3 A	1.95 A	458.8 A	420.9 A	75.8 B	67.2 B	29.4 B	25.3 A	3.88 A	1.76 A	404.6 A	107.2 A	74.8 B	70.6 B	26.3 B	21.7 A
ANOVA by Genotype																	
Water Environment (WE)		<0.0001		0.004		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	
Genotype (G)		<0.0001		0.631		<0.0001		<0.0001		0.402		0.386		<0.0001		<0.0001	
WE x G		0.3398		0.247		0.0017		<0.0001		0.0037		0.264		0.0004		<0.0001	
ANOVA by Drought adaptation grouping																	
Water Environment (WE)		<0.0001		0.007		<0.0001		0.0263		<0.0001		<0.0001		<0.0001		0.0109	
Drought Ad (DA)		0.113		0.241		0.0466		0.0434		0.286		0.2215		0.0025		0.0481	
WE x DA		0.782		0.318		0.1007		0.8654		0.008		0.514		0.2391		0.8262	

Means followed by "a" represent the LSD test results for genotypic differences within each environment, meanwhile "A" represent the LSD test results for differences between drought resistant and drought sensitive lines. Means with the same letter are not significantly different (P =0.05). † Parameters measured at mid-pod fill. ‡ Parameters measured at harvest.

Table 5. Pearson correlation coefficients (r) between grain yield (kg ha^{-1}) and shoot dry weight, shoot Δ^{13}C, grain Δ^{13}C, stomatal conductance (g_s), leaf area index (LAI), pod harvest index (PHI), and hundred seed weight (100 SW) for 14 common bean genotypes grown under well-watered or terminal drought conditions, and across water environments (Overall).

	2012			2013		
	Well-watered	**Terminal Drought**	**Overall**	**Well-watered**	**Terminal Drought**	**Overall**
Shoot dry weight (kg ha^{-1})	-0.047	0.503***	0.398***	0.433***	0.525***	0.747***
Shoot Δ^{13}C (‰)	0.067	0.207	0.580***	-0.028	0.398**	0.698***
Grain Δ^{13}C (‰)	0.450***	0.531***	0.714***	0.315*	0.372**	0.610***
g_s (mol H_2O m^{-2} s^{-1})	0.005	0.039	0.213*	0.109	-0.033	0.679***
LAI (leaf m^2 ground area m^{-2})	0.400**	0.428***	0.68***	0.181	0.424**	0.623***
PHI (%)	0.405**	0.651***	0.715***	0.501***	0.58***	0.575***
100SW (g)	0.115	0.122	0.237*	0.136	-0.133	0.214*

Statistical significance: *, $p \leq 0.05$, **, $p \leq 0.01$, ***, $p \leq 0.001$.

Figure legends

Figure 1. Shoot dry weight at mid pod fill of 14 common bean genotypes planted under well-watered (a and c) and terminal drought stress conditions (b and d) near Palmira (Colombia) in 2012 (a, b), and 2013 (c, d). Classification into drought resistant and drought sensitive lines according to Table 3. Bars represent means ± standard error (n = 4). Genotypes with means with the same lower-case letter are not significantly different based on LSD (P =0.05). Capital letters represent the LSD results for differences between drought resistant and drought sensitive lines; means with the same capital letter are not significantly different (P =0.05). The absence of lower-case letters represents absence of genotypic significance according to Table 2. The absence of capital letters represents absence of significant differences between drought resistant and drought sensitive lines according to Table 2.

Figure 2. Shoot $\Delta^{13}C$ at mid pod filling and grain $\Delta^{13}C$ at maturity of 14 common bean genotypes planted under well-watered (white, a and c) and terminal drought conditions (grey, b and d) at Palmira (Colombia) in 2012 (a and b), and 2013 (b and c). Classification into drought resistant and drought sensitive lines according to Table 3. Bars represent means ± standard error (n = 4). Lower case letters represent the LSD test for genotypic differences within each environment; means showing the same lower-case letter are not significantly different (P =0.05). Capital letters represent the LSD test for differences between drought resistant and drought sensitive lines; means showing the same capital letter are not significantly different (P =0.05).

Figure 3. Relationship between mean values of grain yield and grain $\Delta^{13}C$ (‰) as well as grain yield and shoot $\Delta^{13}C$ (‰) of 14 common bean genotypes planted under terminal drought conditions at Palmira (Colombia) in 2012 and 2013. Red symbols represent drought resistant genotypes, while open symbol represents drought sensitive genotypes. The trend line, Pearson's coefficient, and p-values are shown when the correlation between parameters is significant for all genotypes (black line), and/or for drought resistant genotypes (red line). Vertical bars represent grain yield standard error for each genotype, and horizontal bars represent $\Delta^{13}C$ standard errors for each genotype. Fine stippled horizontal and vertical lines indicate mean grain yield and mean $\Delta^{13}C$ values for all the cultivars. Genotypes with $\Delta^{13}C$ values less than the mean were classified as Water Savers and those with $\Delta^{13}C$ values greater than the mean as Water Spenders.

Figure 4. Relationship between yield drought index and shoot $\Delta^{13}C$ (‰) and yield drought index and grain $\Delta^{13}C$ (‰) of 14 common bean genotypes grown in 2012 and 2013 at Palmira (Colombia). Shoot $\Delta^{13}C$ and grain $\Delta^{13}C$ data are from the terminal drought environment. Red symbols represent genotypes classified as drought resistant, while open symbol represents those classified as drought sensitive. The trend line, Pearson's coefficient, and p-values are shown when the correlation between parameters was significant for the overall genotypes (black line), and for drought resistant genotypes (red line). Vertical bars represent yield drought index standard errors for each genotype for each genotype, and horizontal bars represent $\Delta^{13}C$ standard errors for each genotype.

Supplemental table 1: Pearson correlations between all studied parameters separate by year and water environment.

	Year	Water Availability	Dry Weight Biomass	Biomass CID	Grain CID	Stomatal Conductance	Leaf Area Index (LAI)	Pod Number (m^{-2})	Seed Number (m^{-2})	Pod Harvest Index	Hundred seed weight (g)	Grain Yield
Dry Weight Biomass	**2012**	Overall	1	0.288**	0.34372***	0.117	0.470***	0.425***	0.390***	-0.340**	-0.020	0.398***
		Well-Watered		0.259	0.163	-0.017	0.268	0.202	0.206	0.104	-0.263	-0.047
		Terminal Drought		-0.035	0.18274	0.039	0.461***	0.337**	0.304*	0.239	0.05	0.503***
	2013	Overall	1	0.620***	0.520***	0.586***	0.572***	0.631***	0.568***	0.476***	0.123	0.747***
		Well-Watered		-0.101	0.154	-0.002	0.282*	0.290*	0.262*	0.306*	-0.079	0.433***
		Terminal Drought		0.330*	0.258	-0.036	0.169	0.392**	0.348**	0.394**	-0.043	0.525***
Biomass CID	**2012**	Overall	0.288**	1	0.700***	0.234*	0.490***	0.519***	0.595***	0.581***	-0.195	0.580**
		Well-Watered	0.259		0.499***	-0.009	0.187	0.160	0.331*	0.245	-0.498***	0.207
		Terminal Drought	-0.035		0.472***	0.124	0.009	0.219	0.387**	0.308*	-0.526***	0.618***
	2013	Overall	0.620***	1	0.606***	0.795***	0.507***	0.629***	0.580***	0.393***	0.141	0.698***
		Well-Watered	-0.101		0.410**	0.383**	-0.229	0.19	0.293*	0.102	-0.361***	-0.028
		Terminal Drought	0.330*		0.21	0.149	0.136	0.242	0.191	0.244	0.156	0.398**
Grain CID	**2012**	Overall	0.343***	0.700***	1	0.186*	0.502***	0.660***	0.701***	0.722***	-0.119	0.714***
		Well-Watered	0.163	0.499***		0.140	0.180	0.420**	0.500***	0.608***	-0.334*	0.450***
		Terminal Drought	0.182	0.472***		-0.061	0.209	0.517***	0.638**	0.511***	-0.357**	0.531***
	2013	Overall	0.520***	0.606***	1	0.498***	0.408***	0.515***	0.493***	0.291**	0.125	0.610***
		Well-Watered	0.154	0.410**		0.123	-0.197	0.116	0.232	0.435**	-0.039	0.315*
		Terminal Drought	0.258*	0.215		-0.107	0.256*	0.339**	0.297*	0.008	-0.011	0.372**
Stomatal Conductance	**2012**	Overall	0.117	0.234*	0.186*	1	0.333***	0.143	0.126	0.031	0.065	0.213*
		Well-Watered	-0.017	-0.009	0.140		0.319*	-0.047	-0.094	0.024	0.099	0.005
		Terminal Drought	0.039	0.124	-0.061		0.073	0.012	0.041	-0.296*	-0.103	0.039
	2013	Overall	0.586***	0.795***	0.498***	1	0.535***	0.619***	0.556***	0.317***	0.141	0.679***
		Well-Watered	-0.002	0.383**	0.123		-0.037	0.219	0.217	0.047	-0.149	0.109
		Terminal Drought	-0.036	0.149	-0.107		-0.005	0.017	0.041	0.001	-0.116	-0.033
Leaf Area Index (LAI)	**2012**	Overall	0.470***	0.490***	0.502***	0.333***	1	0.481***	0.489***	0.443***	0.042	0.680***
		Well-Watered	0.268	0.187	0.180	0.319		0.214	0.220	0.050	-0.180	0.400**
		Terminal Drought	0.461***	0.009	0.209	0.073		0.081	0.101	0.189	-0.012	0.428***
	2013	Overall	0.572***	0.507***	0.408***	0.535***	1	0.561***	0.518***	0.321***	0.126	0.623***
		Well-Watered	0.282*	-0.229	-0.19728	-0.037		0.221	0.187	0.001	-0.066	0.181
		Terminal Drought	0.169	0.136	0.256*	-0.005		0.298*	0.347**	0.237	-0.002	0.424**
Pod Number (m^{-2})	**2012**	Overall	0.425***	0.519***	0.660***	0.143	0.481***	1	0.912***	0.588***	-0.237	0.572***
		Well-Watered	0.202	0.160	0.420**	-0.047	0.214		0.862***	0.283*	-0.621***	0.050
		Terminal Drought	0.377**	0.219	0.517***	0.012	0.081		0.893	0.479***	-0.302*	0.469***
	2013	Overall	0.631***	0.629***	0.515***	0.619***	0.561***	1	0.919***	0.512***	-0.165	0.844***
		Well-Watered	0.290*	0.19	0.116	0.219	0.221		0.866***	0.283	-0.450***	0.292*
		Terminal Drought	0.392**	0.242	0.339**	0.017	0.298*		0.903***	0.524***	-0.432***	0.840***
Seed Number (m^{-2})	**2012**	Overall	0.390***	0.595***	0.701***	0.126	0.489***	0.912***	1	0.551***	-0.382***	0.555***
		Well-Watered	0.206	0.331*	0.500***	-0.094	0.220	0.862***		0.230	-0.776***	0.080
		Terminal Drought	0.304*	0.387**	0.638***	0.041	0.101	0.893***		0.497***	-0.416**	0.498***
	2013	Overall	0.568***	0.580***	0.493***	0.556***	0.518***	0.919***	1	0.464***	-0.392***	0.755***
		Well-Watered	0.262*	0.293*	0.232	0.217	0.187	0.866***		0.205	-0.729***	0.491***
		Terminal Drought	0.348**	0.191	0.297*	0.041	0.347**	0.903***		0.496***	-0.578***	0.818***
Pod Harvest Index (PHI)	**2012**	Overall	0.340**	0.581***	0.722***	0.031	0.443***	0.588***	0.551***	1	0.097	0.715**
		Well-Watered	0.104	0.245	0.608***	0.024	0.050	0.283*	0.230		-0.046	0.405**
		Terminal Drought	0.239	0.308*	0.551***	-0.296*	0.189	0.479***	0.497***		-0.018	0.651***
	2013	Overall	0.476***	0.393***	0.291**	0.317***	0.321***	0.512***	0.464***	1	0.021	0.575***
		Well-Watered	0.306*	0.102	0.435**	0.047	0.001	0.283*	0.205		0.001	0.501***
		Terminal Drought	0.394**	0.244	0.008	0.001	0.237	0.524***	0.496***		-0.142	0.580***
Hundred seed weight (g)	**2012**	Overall	-0.020	-0.195*	-0.119	0.065	0.042	-0.237*	-0.382***	0.097	1	0.237*
		Well-Watered	-0.263	-0.498***	-0.334*	0.099	-0.180	-0.621***	-0.776***	-0.046		0.115
		Terminal Drought	0.053	-0.526***	-0.357**	-0.103	-0.012	-0.302*	-0.416**	-0.018		0.122
	2013	Overall	0.123	0.141	0.125	0.141	0.126	-0.165	-0.392***	0.021	1	0.214*
		Well-Watered	-0.079	-0.361***	-0.039	-0.149	-0.066	-0.405***	-0.729***	0.001		0.136
		Terminal Drought	-0.043	0.156	-0.011	-0.116	-0.002	-0.432***	-0.578***	-0.142		-0.133
Grain Yield	**2012**	Overall	0.398***	0.580***	0.714***	0.213*	0.680***	0.572***	0.555***	0.715***	0.237**	1
		Well-Watered	-0.047	0.067	0.450***	0.005	0.400**	0.050	0.080	0.405**	0.115	
		Terminal Drought	0.503***	0.207	0.531***	0.039	0.428***	0.469***	0.498***	0.651***	0.122	
	2013	Overall	0.747***	0.698***	0.610***	0.679***	0.623***	0.844***	0.755***	0.575***	0.214*	1
		Well-Watered	0.433***	-0.028	0.315	0.109	0.181	0.635***	0.491***	0.501***	0.136	
		Terminal Drought	0.525***	0.398**	0.372**	-0.033	0.424**	0.840***	0.818***	0.580***	-0.133	

Statistical significance: *, p ≤ 0.05, **, p ≤ 0.01, ***, p ≤ 0.001.

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Figure 1

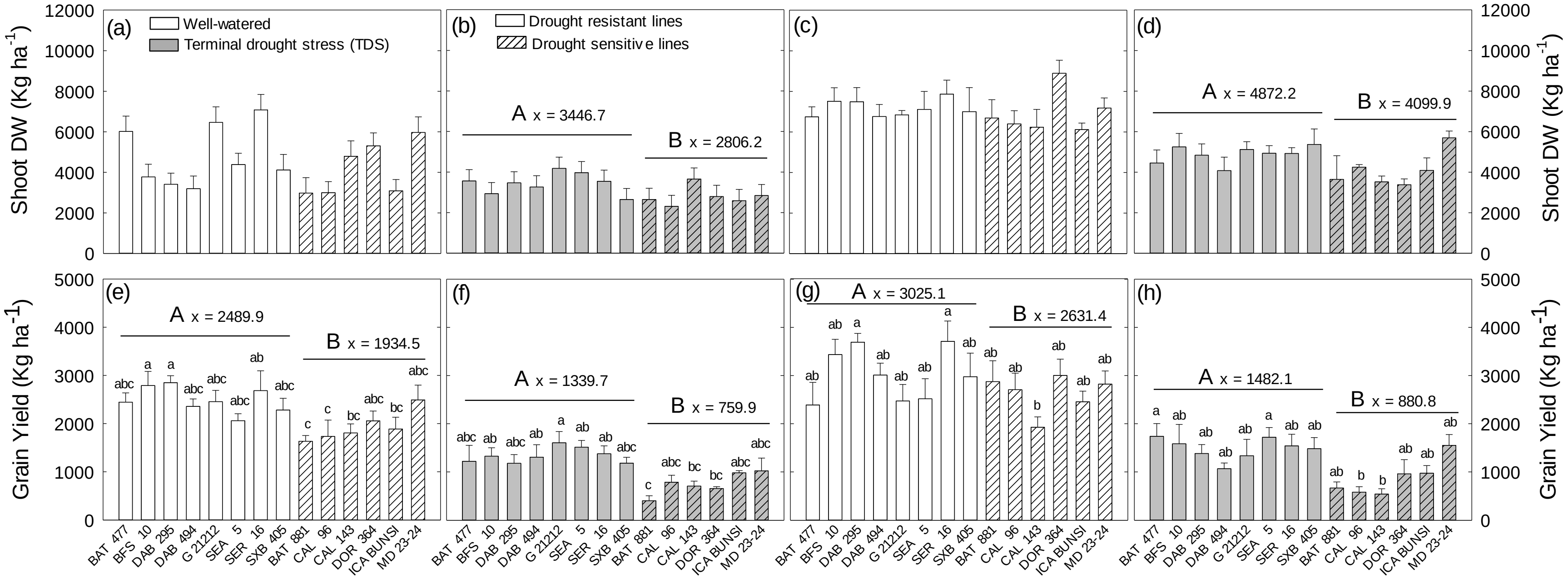

Figure 2

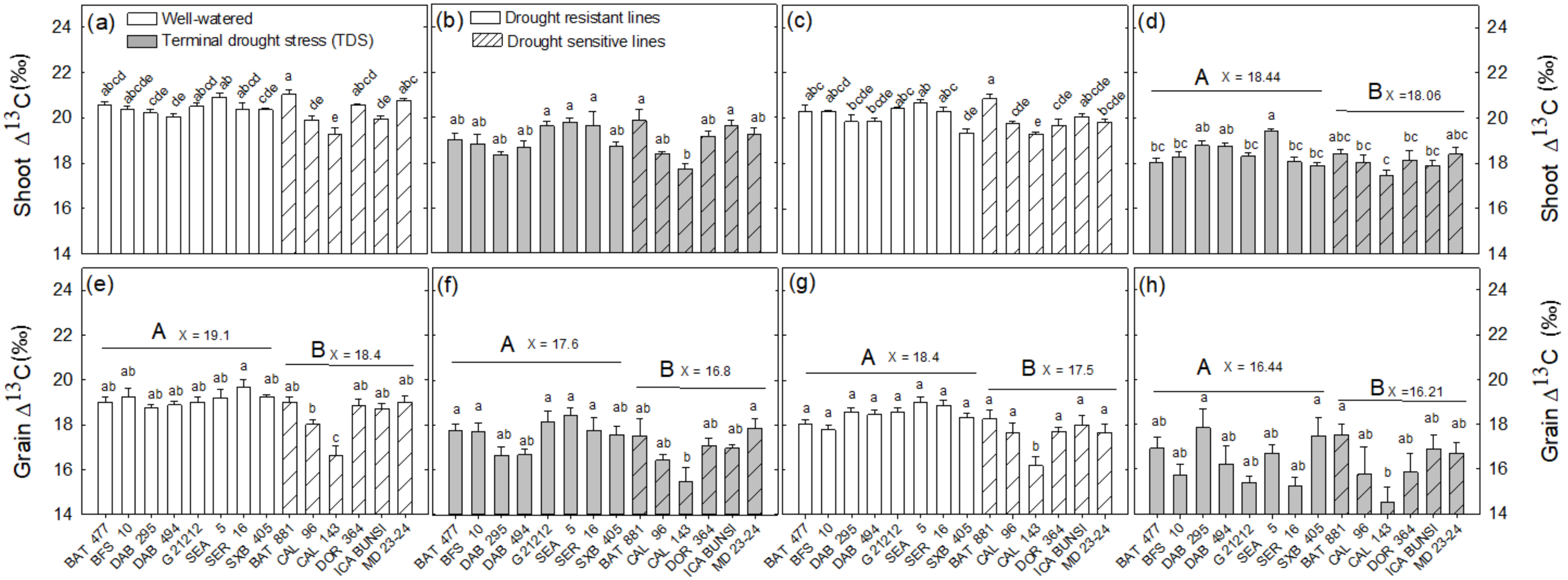
Shoot Δ13C (‰)
Grain Δ13C (‰)
24
22
20
18
16
14
(a)
Well-watered
Terminal drought stress (TDS)
abcd
abcde
cde
de
abcd
ab
abcd
cde
a
de
e
abcd
de
abc
(b)
Drought resistant lines
Drought sensitive lines
ab
ab
ab
ab
a
a
a
ab
a
ab
b
ab
a
ab
(c)
abc
abcd
bcde
bcde
abc
ab
abc
de
a
cde
e
cde
abcde
bcde
(d)
A X = 18.44
B X =18.06
bc
bc
ab
ab
bc
a
bc
bc
abc
bc
c
bc
bc
abc
(e)
A X = 19.1
B X = 18.4
ab
ab
ab
ab
ab
ab
a
ab
ab
b
c
ab
ab
ab
(f)
A X = 17.6
B X = 16.8
a
a
ab
ab
a
a
a
ab
ab
ab
b
ab
ab
a
(g)
A X = 18.4
B X = 17.5
a
a
a
a
a
a
a
a
a
a
b
a
a
a
(h)
A X = 16.44
B X =16.21
ab
ab
a
ab
ab
ab
ab
a
a
ab
b
ab
ab
ab
BAT 477
BFS 10
DAB 295
DAB 494
G 21212
SEA 5
SER 16
SXB 405
BAT 881
CAL 96
CAL 143
DOR 364
ICA BUNSI
MD 23-24

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Figure 3

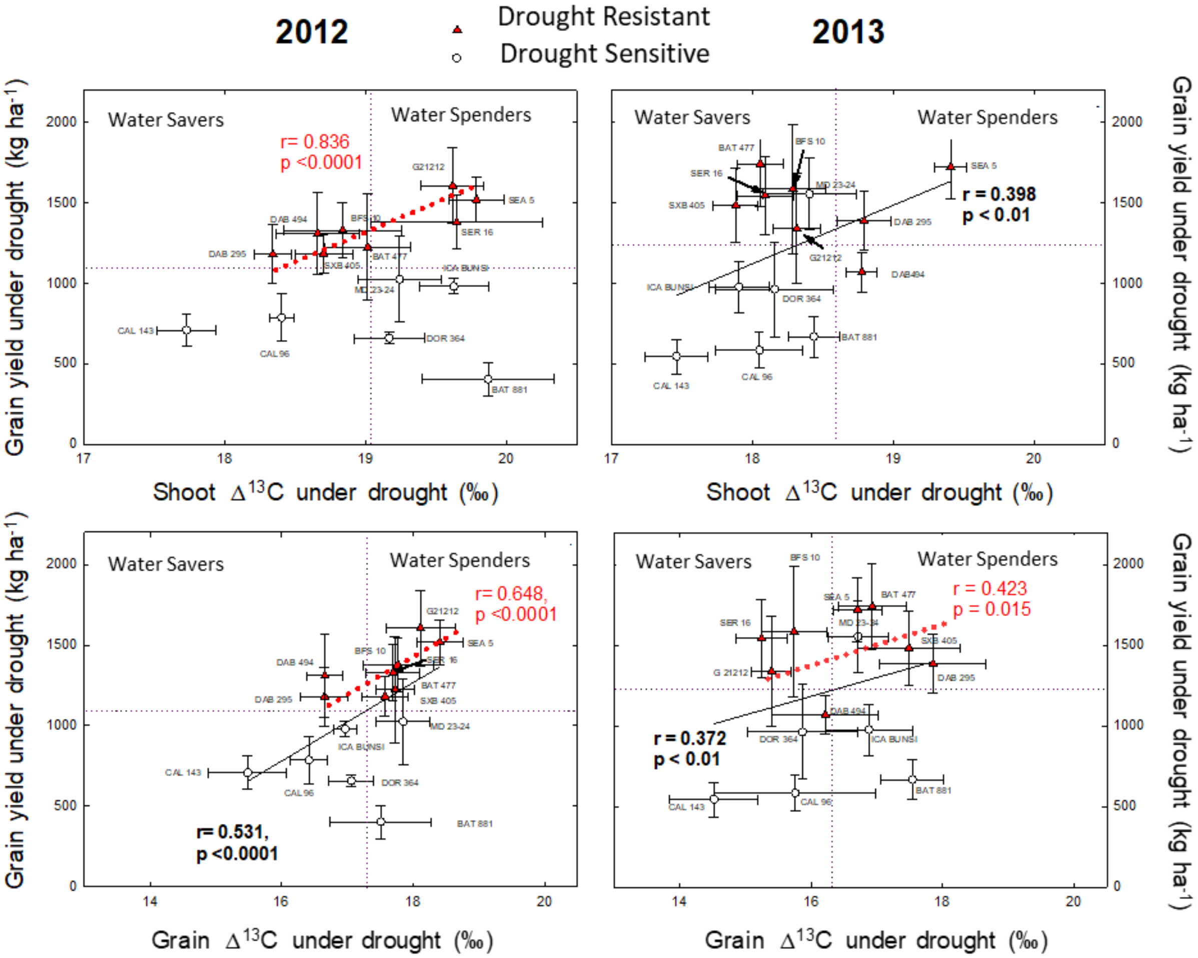
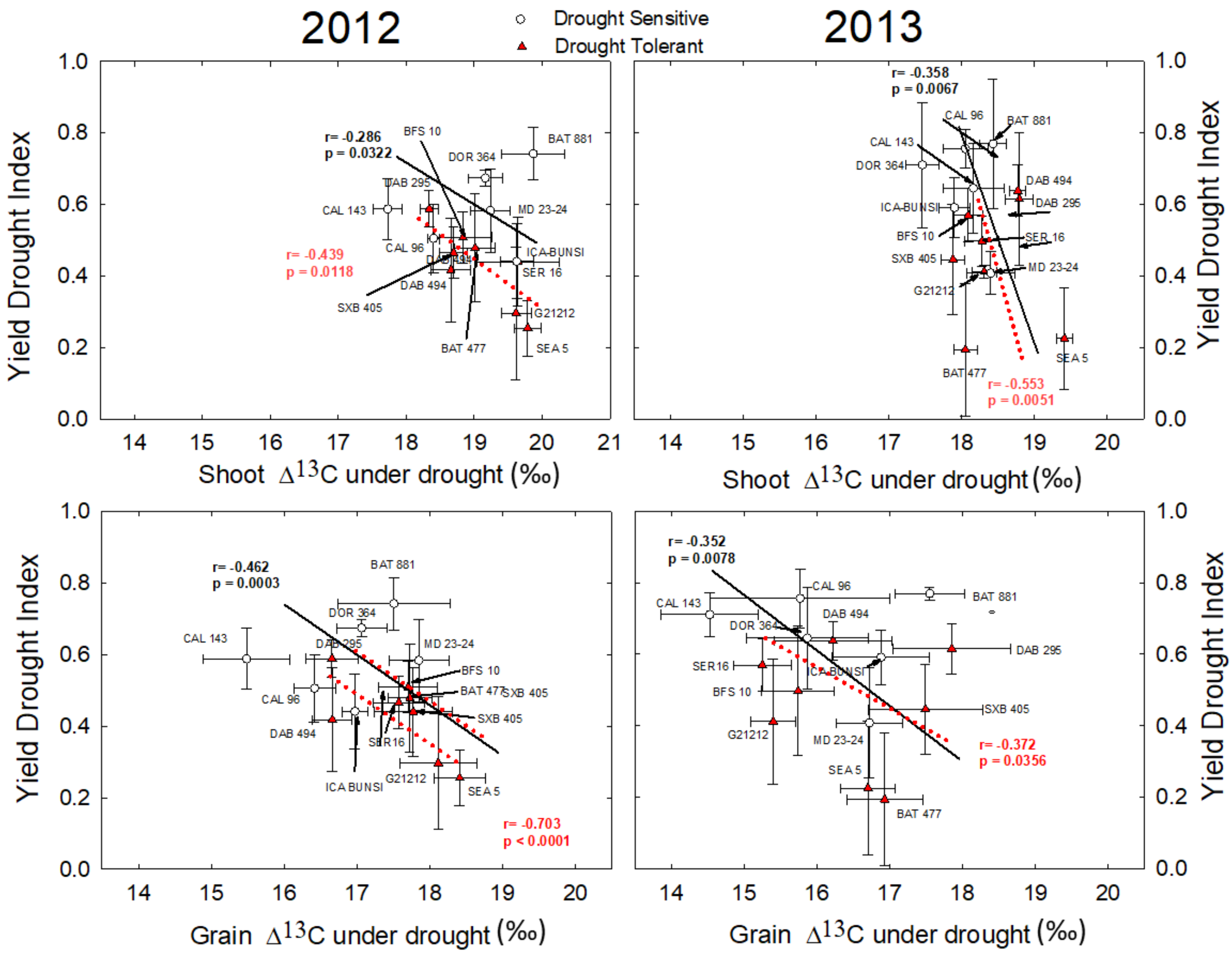


Figure 4


2012
2013
○ Drought Sensitive
▲ Drought Tolerant
Yield Drought Index
Shoot Δ13C under drought (‰)
Grain Δ13C under drought (‰)
r= -0.286
p = 0.0322
r= -0.439
p = 0.0118
r= -0.358
p = 0.0067
r= -0.553
p = 0.0051
r= -0.462
p = 0.0003
r= -0.703
p < 0.0001
r= -0.352
p = 0.0078
r= -0.372
p = 0.0356
BAT 881
BFS 10
DOR 364
DAB 295
CAL 143
MD 23-24
CAL 96
ICA-BUNSI
SER 16
DAB 494
SXB 405
G21212
BAT 477
SEA 5
0.0
0.2
0.4
0.6
0.8
1.0
14
15
16
17
18
19
20
21