

RESEARCH

Using Carbon Isotope Discrimination to Assess Genotypic Differences in Drought Resistance of Parental Lines of Common Bean

Alvaro Sanz-Saez, Michael J.W. Maw, Jose A. Polania, Idupulapati M. Rao, Stephen E. Beebe, and Felix B. Fritsch¹*

ABSTRACT

Accurate assessment of crop water uptake (WU) and water use efficiency (WUE) is not easy under field conditions. Carbon isotope discrimination ($\Delta^{13}\text{C}$) has been used as a surrogate of WUE to examine crop yield responses to drought and its relationship with WU and WUE. A 2-yr study was conducted (i) to characterize genotypic variation in $\Delta^{13}\text{C}$, grain yield, and other physiological parameters in common bean (*Phaseolus vulgaris* L.) parental lines, and (ii) to examine the relationships between grain $\Delta^{13}\text{C}$, shoot $\Delta^{13}\text{C}$, 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 $\Delta^{13}\text{C}$, and grain $\Delta^{13}\text{C}$ 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 (i) greater water uptake, as indicated by high $\Delta^{13}\text{C}$ in genotypes previously shown to have deeper roots (e.g., SEA 5 and BAT 477), and (ii) increased WUE, denoted by lower $\Delta^{13}\text{C}$ and greater pod harvest index (PHI) (e.g., SER 16). Coupling of $\Delta^{13}\text{C}$ 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.

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Abbreviations: DW, dry weight; HI, harvest index; LAI, leaf area index; MPF, mid-pod fill; 100SW, hundred-seed weight; PHI, pod harvest index; TDS, terminal drought stress; WW, well-watered; WU, water uptake; WUE, water use efficiency; YDI, yield drought index.

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 ~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

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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, 2016b).

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, 2017b), 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., 2014).

In crop production, WUE can be defined at various scales. Agronomic WUE is defined as yield per unit of irrigation and/or precipitation (Passioura, 1977), and physiological WUE is defined as the aboveground 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, 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, 1996), are of great interest when breeding cultivars for high yields under water-limited conditions.

Carbon isotope discrimination ($\Delta^{13}\text{C}$) signatures of shoot biomass or seed samples have been demonstrated to be negatively correlated with physiological WUE in several crop species, including common bean (El-Sharkawy et al., 1985; Farquhar et al., 1989; Ehleringer et al., 1991). 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 $\Delta^{13}\text{C}$ signatures and lower WUE. In subsequent work, White et al. (1994a, 1994b) examined the relationship between $\Delta^{13}\text{C}$ and yield in F_2 and F_3 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, Polania et al. (2016a, 2016b) successfully used $\Delta^{13}\text{C}$ of shoot and seed tissues to predict common bean yield response under irrigated and rainfed conditions. Like common bean, observations in other species also indicate complex relationships of $\Delta^{13}\text{C}$ and seed yield (Brito et al., 2014; Vadez and Ratnakumar, 2016). For instance, in wheat (*Triticum aestivum* L.), $\Delta^{13}\text{C}$ 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, $\Delta^{13}\text{C}$ 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 with 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 $\Delta^{13}\text{C}$ 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 morphophysiological 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, 2016b). 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., 2016a; Diaz et al., 2018). However, the genotypic variation of $\Delta^{13}\text{C}$ 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 (i) to characterize genotypic variation in $\Delta^{13}\text{C}$, yield, and other physiological parameters including leaf area index (LAI), g_s , PHI, and hundred-seed weight (100SW) under well-watered (WW) and terminal drought stress (TDS) conditions, and (ii) to 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 asl during the dry seasons (June–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 Hapludolls) 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 and -1.5 MPa as upper and lower limits for soil matric potential, respectively).

Two levels of water supply were applied to impose WW and TDS conditions. Well-watered and TDS 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: (i) significance in the CIAT Mesoamerican and Andean breeding programs, (ii) diversity in genetic backgrounds, and (iii) whether they 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; Singh et al., 1990; White et al., 1990), high HI under drought (SER 16; Polania et al., 2016b),

high shoot vigor under drought (SXB05; Assefa et al., 2013), drought and low-fertility adaptation (BFS10; Beebe et al., 2008; Suarez-Salazar et al., 2019), and genotypes described as drought resistant by CIAT due to their high yields under drought conditions (DAB 295, DAB 494; Polania et al., 2016b; Suarez-Salazar et al., 2019). Drought-sensitive lines were selected for their commercial use and known sensitivity to drought (Assefa et al., 2013; Polania et al., 2016a, 2017a; 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 wide 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 1 wk 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 five times in 2012 and six times in 2013 with 35 mm of water per irrigation.

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

Year	Avg. daily max. temperature	Avg. daily min. 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	185.0	255.0	373.3	1185.3
2013	29.9 ± 2.0	19.1 ± 1.0	183.1	288.1	333.2	1141.3

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	YDI		Characteristics of selection of each cultivar and references
		2012	2013	
Drought resistant	BAT 477	0.479abc†	0.194c	Deep root (White et al., 1990)
	BFS 10	0.508abc	0.497abc	Low fertility and drought adapted (Suarez-Salazar et al., 2019)
	DAB 295	0.588ab	0.615ab	Drought adapted
	DAB 494	0.418bc	0.638ab	Drought adapted
	G 21212	0.296c	0.412bc	Low fertility adapted (Beebe et al., 2008)
	SEA 5	0.254c	0.226c	Deep root (Beebe et al., 2014)
	SER 16	0.440bc	0.569ab	High harvest index (Polania et al., 2016b)
	SXB 405	0.465bc	0.446abc	Drought adapted (Assefa et al., 2013)
	Mean	0.431B	0.449B	
Drought sensitive	BAT 881	0.742a	0.769a	Commercial line (Diaz et al., 2018)
	CAL 96	0.505abc	0.756a	Commercial line (Polania et al., 2016a)
	CAL 143	0.587ab	0.710ab	Commercial line (Polania et al., 2016a)
	DOR 364	0.674ab	0.645ab	Commercial line (Polania et al., 2016a)
	ICA BUNSI	0.441bc	0.591ab	Commercial line (Assefa et al., 2013)
	MD 23–24	0.583ab	0.408bc	Commercial line (Polania et al., 2017a)
	Mean	0.589A	0.647A	
		<i>p</i> values		
ANOVA effects by		2012	2013	
Genotype (G)		0.078	0.022	
Drought adaptation (DA)		0.005	0.006	

† Means followed by lowercase letters represent the LSD test results for genotypic differences within each environment. Uppercase letters represent the LSD test results for differences between drought resistant and drought sensitive lines. Means followed by a common letter are not significantly different from each other ($p = 0.05$).

In both growing seasons, no fertilizer applications were made, but fields were managed with herbicides to control weeds (fomesafen, fluazifop-p-butyl, 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 >30 yr, 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 (third or fourth leaf from the shoot apex) was measured per plot using a leaf porometer (Decagon SC-1, Meter Environment). Measurements were performed between 1100 and 1300 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 ~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) and used to calculate 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

$$YDI = (\text{Yield}_{\text{WW}} - \text{Yield}_{\text{TD}}) / \text{Yield}_{\text{TD}}$$

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

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 University of California Davis Stable Isotopes Facility (Davis, CA) for C isotope analysis. Samples were analyzed using an isotope ratio mass spectrometer (IsoPrime, Elementar France) coupled to an elemental analyzer (EA3000, EuroVector). The $^{13}\text{C}/^{12}\text{C}$ ratio (R) in plant material was first calculated in δ notation ($\delta^{13}\text{C}$, C 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 ($\Delta^{13}\text{C}$, ‰):

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

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

$$\Delta^{13}\text{C} = (\delta^{13}\text{C}_{\text{atm}} - \delta^{13}\text{C}_{\text{sample}}) / (\delta^{13}\text{C}_{\text{sample}} + 1)$$

where $\delta^{13}\text{C}_{\text{atm}}$ is the C isotope composition of atmospheric CO_2 (−8‰; Farquhar et al., 1989) and $\delta^{13}\text{C}_{\text{sample}}$ is the C isotope composition of the plant sample (aboveground biomass or seed).

Statistical Analysis

To determine if genotypes differed in the measured traits under different water availability, mixed model ANOVA using PROC MIXED in SAS 9.4 (SAS Institute) was performed with year, genotype, and water availability (later called environment) modeled 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 locations) in the analysis, and the environment \times 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 YDI, with genotype as a fixed effect and replicate as a 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, dry weight (DW), shoot $\Delta^{13}\text{C}$, grain $\Delta^{13}\text{C}$, 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 \times environment interactions. In 2013, genotype was significant for all but three parameters (shoot DW, LAI, and g_s ; Table 3), and the genotype \times environment interaction was significant for shoot $\Delta^{13}\text{C}$, 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 in the 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 with 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.

Table 3. Analysis of variance (p value) for grain yield, grain C isotope discrimination ($\Delta^{13}\text{C}$), shoot dry weight at mid-pod filling (MPF), and shoot $\Delta^{13}\text{C}$ 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 $\Delta^{13}\text{C}$	Shoot DW†	Shoot $\Delta^{13}\text{C}$
ANOVA by genotypes					
2012	Environment (E)	<0.0001	<0.0001	0.137	<0.0001
	Genotype (G)	<0.0001	<0.0001	0.413	<0.0001
	E \times 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 \times 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 \times 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 \times DA	0.398	0.149	0.295	0.438

† DW, dry weight.

Environment and genotype effects were highly significant for both shoot $\Delta^{13}\text{C}$ and grain $\Delta^{13}\text{C}$ in both years (Table 3, Fig. 2). Genotype \times environment interactions were not significant in 2012, and in 2013 the interaction effect was significant for shoot $\Delta^{13}\text{C}$ only. On average, shoot $\Delta^{13}\text{C}$ in the TDS environment was reduced by 6.4 (2012) and 8.8% (2013) in comparison with the WW environments. Similarly, grain $\Delta^{13}\text{C}$ in the TDS environments was reduced by 8.1 (2012) and 9.6% (2013) compared with the values determined for the WW environments. Correlation analyses between shoot $\Delta^{13}\text{C}$ and grain $\Delta^{13}\text{C}$ from both TDS and WW environments showed significant positive relationships in 2012 ($r = 0.70$, ≤ 0.001) and 2013 ($r = 0.61$, $p \leq 0.001$). When examined by environment, correlations between shoot $\Delta^{13}\text{C}$ and grain $\Delta^{13}\text{C}$ were significant in both environments in 2012, but only in TDS in 2013 (Supplemental Table S1). In both years and environments, average shoot $\Delta^{13}\text{C}$ was greater than average grain $\Delta^{13}\text{C}$ (Fig. 2), but the environment did not appear to influence the relationship between shoot $\Delta^{13}\text{C}$ and grain $\Delta^{13}\text{C}$ in that the shoot/grain $\Delta^{13}\text{C}$ 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 \times 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 \times 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 with 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 \times 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 or drought sensitive 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 $\Delta^{13}\text{C}$ 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 drought-sensitive genotypes exhibiting greater YDIs than drought-resistant genotypes (Table 3). Drought-resistant genotypes showed greater grain yield,

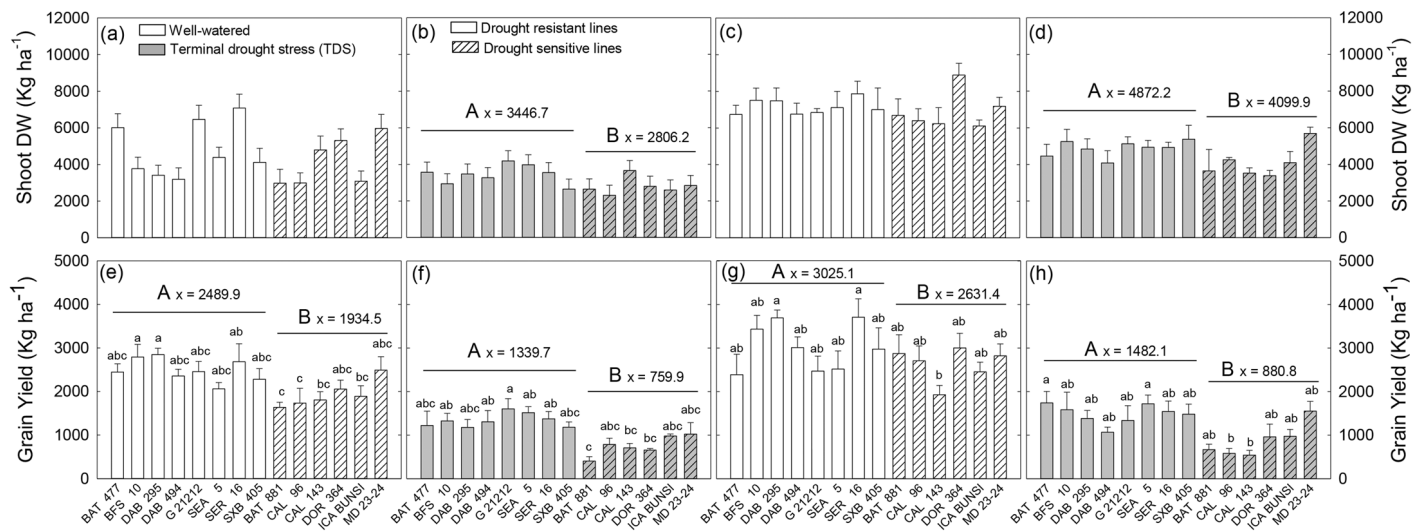


Fig. 1. Shoot dry weight (DW) at mid-pod fill of 14 common bean genotypes planted under (a and c) well-watered and (b and d) terminal drought stress conditions near Palmira (Colombia) in (a, b) 2012 and (c, d) 2013. Classification into drought-resistant and drought-sensitive lines according to Table 3. Bars represent means \pm standard error ($n = 4$). Genotypes with means with a common lowercase letter are not significantly different based on LSD ($p = 0.05$). Uppercase letters represent the LSD results for differences between drought-resistant and drought-sensitive lines; means with a common uppercase letter are not significantly different ($p = 0.05$). The absence of lowercase 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.

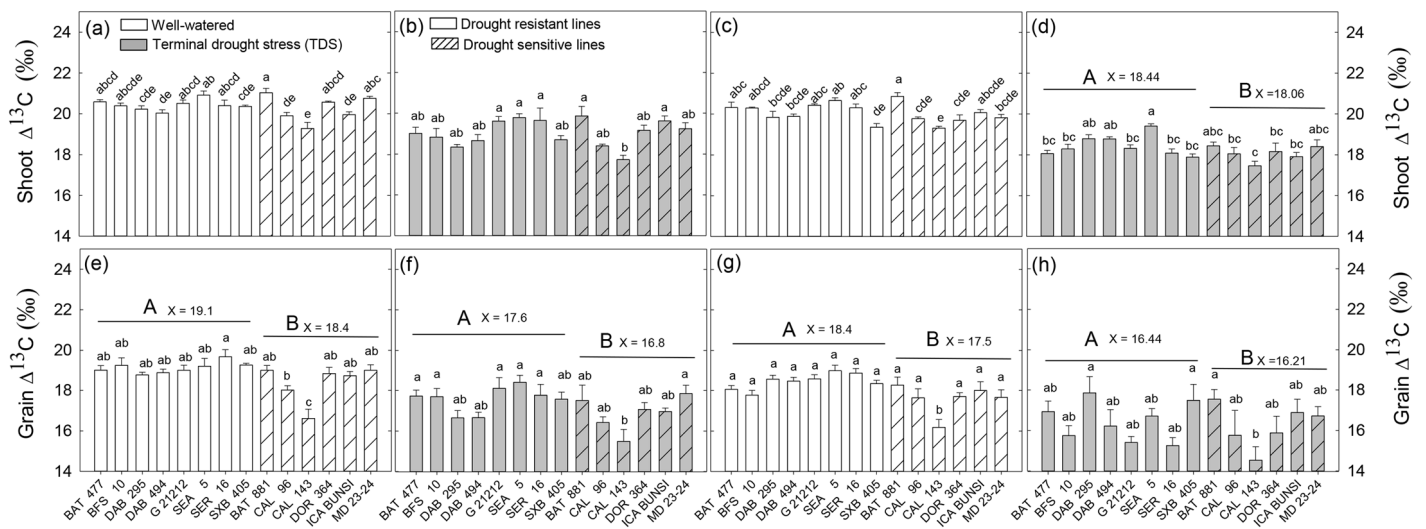


Fig. 2. Shoot C isotope discrimination ($\Delta^{13}\text{C}$) at mid-pod filling and grain $\Delta^{13}\text{C}$ at maturity of 14 common bean genotypes planted under (a and c) well-watered (white) and (b and d) terminal drought conditions (gray) at Palmira (Colombia) in (a and b) 2012 and (b and c) 2013. Classification into drought resistant and drought sensitive lines according to Table 3. Bars represent means \pm standard error ($n = 4$). Lowercase letters represent the LSD test for genotypic differences within each environment; means with a common lowercase letter are not significantly different ($p = 0.05$). Uppercase letters represent the LSD test for differences between drought resistant and drought sensitive lines; means with a common capital letter are not significantly different ($p = 0.05$).

grain $\Delta^{13}\text{C}$, and PHI in both years and in both WW and TDS environments compared with the drought-sensitive genotypes (Tables 3 and 4, Fig. 1 and 2). Additionally, in the TDS environment, drought-resistant genotypes displayed greater shoot DW than drought-sensitive genotypes in both years. For all other traits (shoot $\Delta^{13}\text{C}$, LAI, g_s , and 100SW), the differences between the drought-resistant and drought-sensitive groups were either not significant or only significant in 1 of the 2 yr.

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 $\Delta^{13}\text{C}$ 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 1 of

Table 4. Leaf area index (LAI), stomatal conductance (g_s), pod harvest index (PHI), and hundred-seed weight (100 SW) mean values of 14 common bean genotypes grown under well-watered (WW) and terminal drought stress (TDS) conditions in 2012 and 2013, and ANOVA (p value) for the same parameters measured in 201, 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	2012						2013								
	LAI†			g _s †			100SW‡			PHI‡					
	WW	TDS	WW	WW	TDS	PHI‡	WW	TDS	WW	TDS	PHI‡	WW	TDS	PHI‡	
Drought resistant	Line														
Drought sensitive	BAT 477	4.35ab§	2.38abcd	425.40	368.70	74.7cd	70.3abcd	24.4fe	21.4f	2.86d	2.95a	72.3de	72.7b	19.2hi	17.7de
	BFS 10	3.2cd	1.87cdef	446.20	292.65	79.4ab	76.3a	29.1d	25.9cde	3.77abcd	2.26abc	446.97	92.60	78.9a	79.2a
	DAB 295	3.36cd	1.89cdef	453.08	384.28	75.7bcd	72.2abcd	51.1b	39.5a	3.44bcd	2.71ab	360.02	55.76	75.3abcd	71.2bc
	DAB 494	2.21fe	1.81ef	455.90	390.50	78.4abc	72.4abcd	49.2b	40.9a	2.9d	1.62c	375.50	69.50	76.6abc	71.3bc
	G 21212	4.48a	2.62ab	433.60	401.73	79.2ab	74.6ab	30.2d	27.2cd	2.66d	2.66ab	348.22	57.45	77.1abc	74.1ab
	SEA 5	3.94abc	2.88a	582.43	318.45	76.9bcd	75.2ab	28.8d	25.8cde	3.5bcd	2.04abc	359.30	111.48	76.3abc	75.7ab
	SER 16	3.13cde	1.82ef	499.58	401.15	82a	76.6a	28.1de	23.3def	3.84abcd	1.87bc	425.20	74.73	79.1a	76.4ab
	SXB 405	3.16cde	2.36abcde	343.88	431.38	77.7bcd	68.4bcde	29.7d	28.3c	3.31bcd	1.9bc	376.78	49.63	76.6abc	76.3ab
	Mean	3.48A	2.21A	455A	373.6A	77.7A	68.4A	33.8A	29.4A	3.29B	2.43A	394.2A	73.1A	76.5A	74.6A
															25.1A
Drought sensitive	BAT 881	3.35cd	1.81ef	522.68	487.73	74.3de	65.4de	20.5gh	16.2g	4.09abc	1.66c	525.12	104.75	74.1cde	66.1cd
	CAL 96	2.94de	1.81ef	501.28	370.23	78.4abc	68.2bcde	56.7a	43.6a	2.94cd	1.88bc	461.65	68.28	76.3abc	70.3bc
	CAL 143	3.18cde	2.4abc	473.48	439.83	70.6e	60.6e	41.6c	34.4b	4.79a	1.74bc	259.65	131.10	70.6e	64.2d
	DOR 364	3.42bcd	1.84def	410.03	388.45	76.5bcd	66.1cde	22.4fg	21.3f	4.35abc	1.78bc	377.42	91.10	74.8bcd	73.4ab
	ICA BUNSI	2.98cde	1.69f	410.40	499.58	76.6bcd	68.3bcde	18.4h	14.8g	3.38bcd	2.32abc	370.77	115.45	74.4cd	72.3bc
	MD 23–24	3.92abc	2.15bcdef	434.93	339.58	78.6ab	74.3abc	23.9fg	21.9ef	3.71abcd	2.61abc	433.23	132.70	78.6ab	77.3ab
	Mean	3.3A	1.95A	458.8A	420.9A	75.8B	67.2B	29.4B	25.3A	3.88A	1.76A	404.6A	107.2A	74.8B	70.6B
															26.3B
															21.7A
Effect		LAI		g _s		PHI		100SW		LAI		g _s		PHI	
	Water environment (WE)		<0.0001		0.004		<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
	WE × G		0.3398		0.247		0.0017		<0.0001		0.0037		0.264		0.0004
Effect		LAI		g _s		PHI		100SW		LAI		g _s		PHI	
	Water environment (WE)		<0.0001		0.007		<0.0001		0.0263		<0.0001		<0.0001		<0.0001
	Drought adaptation (DA)		0.113		0.241		0.0466		0.0434		0.286		0.2215		0.0025
	WE × DA		0.782		0.318		0.1007		0.8654		0.008		0.514		0.2391

† Parameters measured at mid-pod fill.

‡ Parameters measured at harvest.

§ Means followed by lowercase letters represent the LSD test results for genotypic differences within each environment, whereas uppercase letters represents the LSD test results for differences between drought-resistant and drought-sensitive lines. Means with a common letter are not significantly different ($p = 0.05$).

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

Trait	2012			2013		
	Well-watered	Terminal drought	Overall	Well-watered	Terminal drought	Overall
Shoot dry weight	−0.047	0.503***	0.398***	0.433***	0.525***	0.747***
Shoot $\Delta^{13}\text{C}$	0.067	0.207	0.580***	−0.028	0.398**	0.698***
Grain $\Delta^{13}\text{C}$	0.450***	0.531***	0.714***	0.315*	0.372**	0.610***
g_s	0.005	0.039	0.213*	0.109	−0.033	0.679***
LAI	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	0.115	0.122	0.237*	0.136	−0.133	0.214*

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

the 2 yr. For instance, when separated by environment, shoot $\Delta^{13}\text{C}$ 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 $\Delta^{13}\text{C}$ signature, the mean yield of each genotype in TDS was plotted against grain $\Delta^{13}\text{C}$ and shoot $\Delta^{13}\text{C}$ by year (Fig. 3). Grain yield was positively correlated with shoot $\Delta^{13}\text{C}$ in 2013 but was not correlated with shoot $\Delta^{13}\text{C}$ 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 $\Delta^{13}\text{C}$ for drought-resistant but not drought-sensitive genotypes in 2012. Genotype SEA 5 exhibited the highest shoot $\Delta^{13}\text{C}$ and high grain yield in 2013, and SEA 5 and G21212 had high shoot $\Delta^{13}\text{C}$ and grain yield in 2012 (Fig. 3). Grain yield was positively correlated with grain $\Delta^{13}\text{C}$ when analyzed for all genotypes, and when the genotypes were pooled by drought adaptation, the grain yield was positively correlated with grain $\Delta^{13}\text{C}$ for the drought-resistant genotypes in both years (Fig. 3). Again, genotypes SEA 5 and G21212 exhibited high grain yield and high grain $\Delta^{13}\text{C}$ in 2012 (Fig. 3). In contrast, CAL 143 was consistently low in grain yield and in grain $\Delta^{13}\text{C}$ and shoot $\Delta^{13}\text{C}$.

To explore the utility of C isotope discrimination as a tool to predict yield and yield losses under drought conditions, the relationship between YDI and shoot $\Delta^{13}\text{C}$ and grain $\Delta^{13}\text{C}$ 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 $\Delta^{13}\text{C}$ and grain $\Delta^{13}\text{C}$ in both years, indicating that the genotypes with higher shoot $\Delta^{13}\text{C}$ and grain $\Delta^{13}\text{C}$ under drought maintained greater yields. When the genotypes were separated by drought adaptation group, the drought-resistant genotypes also showed negative correlation between YDI and shoot $\Delta^{13}\text{C}$, but no significant relationship was found for those in the drought-sensitive group (Fig. 4). SEA 5 was the genotype

with the lowest YDI and highest shoot $\Delta^{13}\text{C}$ in both years (Fig. 4), but it exhibited the highest grain $\Delta^{13}\text{C}$ only in 2012. In 2013, BAT477 and SEA 5 had the lowest YDI and similar grain $\Delta^{13}\text{C}$, which was lower than the grain $\Delta^{13}\text{C}$ of other genotypes (DAB 295, SXB 405, and BAT 881) with higher YDI (Table 2, Fig. 4).

DISCUSSION

Impact of Water Availability on Yield, and Physiological and Morphological Traits

Genotypic differences in $\Delta^{13}\text{C}$ of shoot DW harvested at MPF, as well as in grain $\Delta^{13}\text{C}$, were found in both years and in WW and TDS environments (Table 3, Fig. 2). Plants grown under water-limited conditions exhibited lower $\Delta^{13}\text{C}$ values than plants grown under irrigated conditions, which is consistent with lower WUE of WW plants compared with drought-stressed plants observed in previous common bean experiments (White et al., 1994a, 1994b; Beebe et al., 2013; Polania et al., 2016b). The absence of significant genotype \times environment interactions for grain $\Delta^{13}\text{C}$ in either of the 2 yr indicates that the genotypes examined in this study responded similarly under WW and TDS conditions. Interestingly, there was a genotype \times environment interaction for shoot $\Delta^{13}\text{C}$ in 2013. It is unclear why this did not translate to a significant genotype \times environment interaction for grain $\Delta^{13}\text{C}$, but the differences in shoot $\Delta^{13}\text{C}$ 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). Nonetheless, shoot $\Delta^{13}\text{C}$ and grain $\Delta^{13}\text{C}$ revealed significant positive correlations when analyzed across WW and TDS environments in each of the 2 yr ($r = 0.70$ and $r = 0.61$). Interestingly, the correlation coefficients for 2012 and 2013 were comparable with those reported by Kaler et al. (2018) for soybean [*Glycine max* (L.) Merr.] in which shoot $\Delta^{13}\text{C}$ and grain $\Delta^{13}\text{C}$ from rainfed experiments conducted at three locations exhibited a strong relationship ($r = 0.69$).

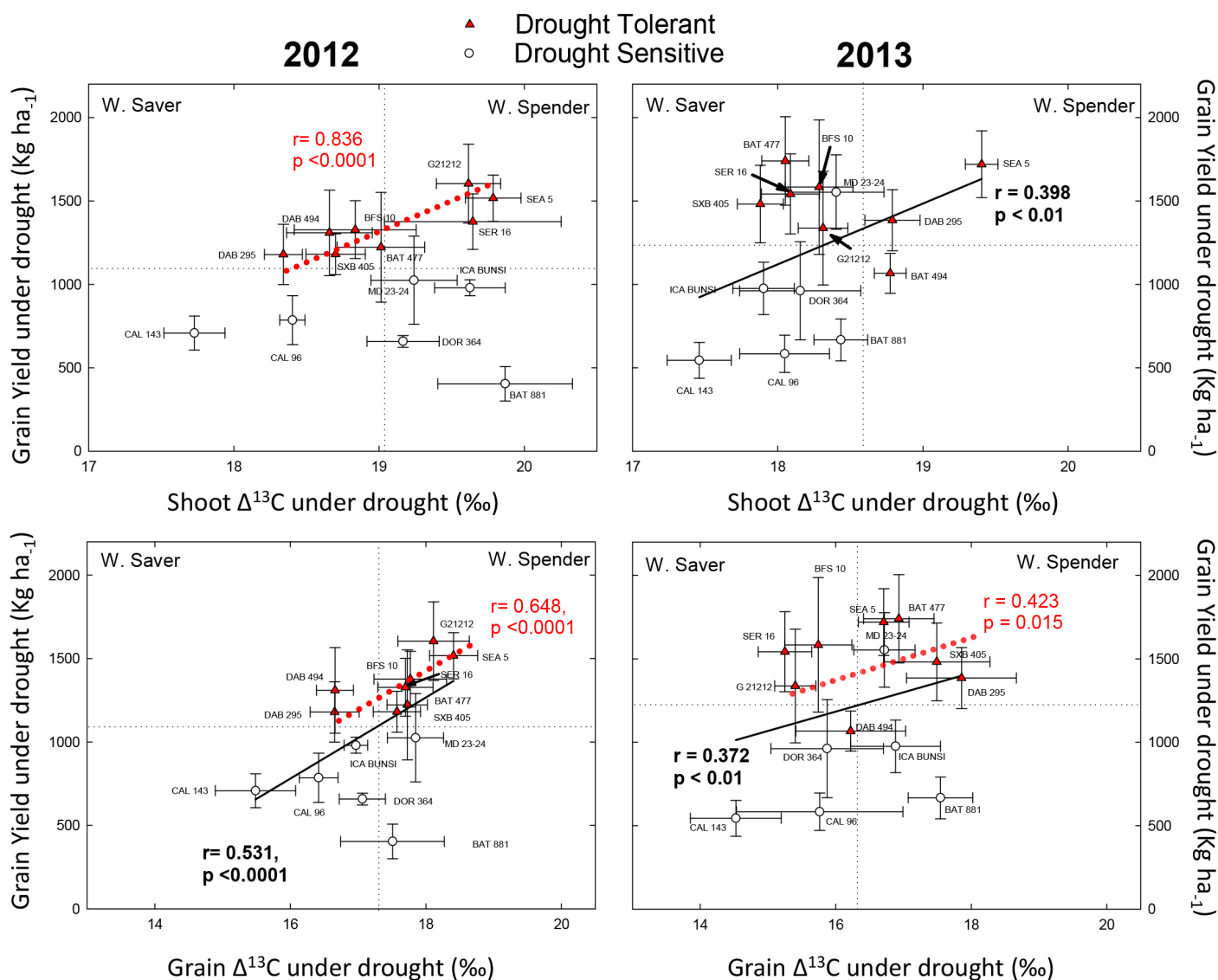


Fig. 3. Relationship between mean values of grain yield and grain C isotope discrimination ($\Delta^{13}\text{C}$) (‰) as well as grain yield and shoot $\Delta^{13}\text{C}$ (‰) of 14 common bean genotypes planted under terminal drought conditions at Palmira (Colombia) in 2012 and 2013. Red symbols represent drought-resistant genotypes, whereas 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 $\Delta^{13}\text{C}$ standard errors for each genotype. Fine stippled horizontal and vertical lines indicate mean grain yield and mean $\Delta^{13}\text{C}$ values for all the cultivars. Genotypes with $\Delta^{13}\text{C}$ values less than the mean were classified as water savers, and those with $\Delta^{13}\text{C}$ values greater than the mean as water spenders.

Unlike soybean (Kaler et al., 2018), the genotypic variation in the present study was greater for shoot $\Delta^{13}\text{C}$ ($F = 8.47$) than grain $\Delta^{13}\text{C}$ ($F = 6.05$). However, as in Kaler et al. (2018), the effect of environment was stronger for shoot $\Delta^{13}\text{C}$ ($F = 361.5$) than for grain $\Delta^{13}\text{C}$ ($F = 103.9$).

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, 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 2 yr. Previously, Polania

et al. (2016b) 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 $\Delta^{13}\text{C}$, 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 with WW environments when averaged across genotypes and years. This is comparable

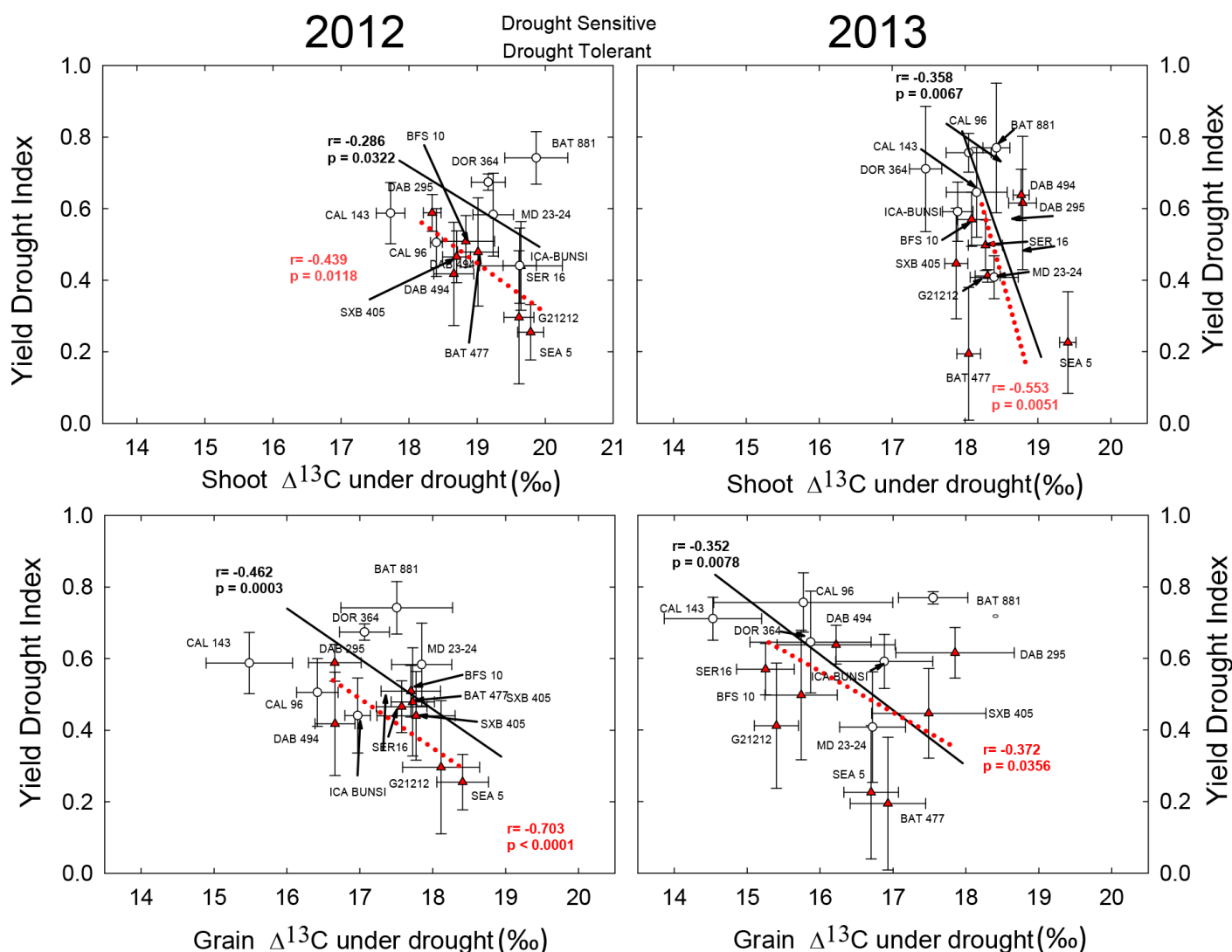


Fig. 4. Relationship between yield drought index and shoot C isotope discrimination ($\Delta^{13}\text{C}$) (‰) and yield drought index and grain $\Delta^{13}\text{C}$ (‰) of 14 common bean genotypes grown in 2012 and 2013 at Palmira (Colombia). Shoot $\Delta^{13}\text{C}$ and grain $\Delta^{13}\text{C}$ data are from the terminal drought environment. Red symbols represent genotypes classified as drought resistant, whereas open symbols represent 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 $\Delta^{13}\text{C}$ standard errors for each genotype.

with the 31 and 36% impact of TDS previously reported by Polania et al. (2016b) and Polania et al. (2017a), respectively. As observed for g_s , genotypic differences in shoot DW were not consistently observed. However, the drought-sensitive genotypes accumulated less (17%) shoot DW than the drought-resistant 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 (Assefa et al., 2013; Rao 2014; Polania et al., 2017a, 2017b).

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., 2017b). 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 \times 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., 2017a). 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 $\sim 39\%$ 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 associated 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 played a role.

As expected, grain yields were significantly lower in TDS compared with WW environments (Fig. 1, Table 3). Although genotype \times environment interactions were not significant in either year, previous studies provide support for the greater average yield of drought-resistant genotypes (51% of WW) than those classified as drought sensitive (36% of WW) under TDS in this study (Fig. 1, see Table 2 for references regarding drought-resistant cultivars). Indeed, grain yields of drought-resistant genotypes were 1.7-fold those of some drought-sensitive 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 greater 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 drought-sensitive and drought-resistant 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 $\Delta^{13}\text{C}$ (White et al., 1990; 1994a,

1994b) to positive relationships only in rainfed conditions (Polania et al., 2016b). In the present study, positive relationships between shoot $\Delta^{13}\text{C}$ and grain yield and between grain $\Delta^{13}\text{C}$ and grain yield were found when examined across TDS and WW environments (Table 5). However, when the relationships between $\Delta^{13}\text{C}$ and grain yield were examined separately by environment, only the grain $\Delta^{13}\text{C}$ showed a positive relationship with grain yield under both water regimes (Fig. 3). These differences in the relationship between $\Delta^{13}\text{C}$ 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., 2016b). Given that shoot samples were collected earlier in the season in comparison with grain samples and with respect to the TDS imposition, the closer relationship of grain $\Delta^{13}\text{C}$ to grain yield was not surprising and was consistent with the $\Delta^{13}\text{C}$ signature integrating the plant response to environmental conditions over different time periods (Farquhar et al., 1989; Condon et al., 2004). Indeed, at the time of shoot sampling (MPF), the plants had not experienced the full extent of the TDS environment, as indicated by the greater relative impact of the TDS on grain yield compared with shoot DW at MPF (Fig. 1). In addition, the lower $\Delta^{13}\text{C}$ value in grain in comparison with shoot tissue has been related with partitioning of C from the shoot to the grain (Polania et al., 2016b). Thus, grain $\Delta^{13}\text{C}$ may be better suited for prediction of yield under drought than shoot $\Delta^{13}\text{C}$ (Polania et al., 2016b). Although the relationship of shoot $\Delta^{13}\text{C}$ with grain yield was less robust than that of grain $\Delta^{13}\text{C}$, leaf or shoot $\Delta^{13}\text{C}$ 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 $\Delta^{13}\text{C}$ 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, 2015). Blum (2009) suggested that lines with high vs. low effective use of water have greater access to water through deeper roots or other mechanisms that allow a more favorable plant water status, and thus they can fix more C through photosynthesis and exhibit higher $\Delta^{13}\text{C}$ signatures. Accordingly, plant varieties may be classified into anisohydric (water spenders) and isohydric (water savers) types (Blum, 2015). The genotypes that had greater grain yield under TDS in this study were classified into water spenders (high grain yield and high $\Delta^{13}\text{C}$) and water savers (high grain yield and low $\Delta^{13}\text{C}$), depending on shoot $\Delta^{13}\text{C}$ and grain $\Delta^{13}\text{C}$ and the corresponding relationships to grain yield (Fig. 3). When yield was compared with grain $\Delta^{13}\text{C}$, only SEA 5 and BAT 477

were consistently classified as water spenders, and SER 16 as a 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 that 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., 2016b).

Shoot $\Delta^{13}\text{C}$ and grain $\Delta^{13}\text{C}$ also have been used to predict yield losses under drought conditions by studying relationships between $\Delta^{13}\text{C}$ and different drought indices (Ehleringer et al., 1991, Polania et al., 2016b). In the present study, YDI was negatively correlated with shoot $\Delta^{13}\text{C}$, which indicates that genotypes that were better able to maintain yields under drought had greater $\Delta^{13}\text{C}$, 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 greater 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 $\Delta^{13}\text{C}$, indicative of high WUE.

CONCLUSIONS

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

Supplemental Material

Supplemental material is available online for this article.

Conflict of Interest

The authors declare that there is no conflict of interest.

Acknowledgments

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References

- Araus, J.L., T. Amaro, J. Casadesus, A. Asbati, and M.M. Nachit. 1998. Relationship between ash content, carbon isotope discrimination and yield in durum wheat. *Aust. J. Plant Physiol.* 25:835–842.
- Araus, J.L., G.A. Slafer, M.P. Reynolds, and C. Royo. 2002. Plant breeding and drought in C_3 cereals: What should we breed for? *Ann. Bot.* 89:925–940. doi:10.1093/aob/mcf049
- Araus, J.L., G.A. Slafer, C. Royo, and M.D. Serret. 2008. Breeding for yield potential and stress adaptation in cereals. *Crit. Rev. Plant Sci.* 27:377–412. doi:10.1080/07352680802467736
- Assefa, T., S. Beebe, I.M. Rao, J. Cuasquer, M.C. Duque, and M. Rivera. 2013. Pod harvest index as a selection criterion to improve drought resistance in white pea bean. *Field Crops Res.* 148:24–33. doi:10.1016/j.fcr.2013.04.008
- Beebe, S. 2012. Common bean breeding in the tropics. *Plant Breed. Rev.* 36:357–426. doi:10.1002/9781118358566.ch5
- Beebe, S., I.M. Rao, M.W. Blair, and J.A. Acosta-Gallegos. 2013. Phenotyping common beans for adaptation to drought. *Front. Plant Sci.* 4:35. doi:10.3389/fphys.2013.00035
- Beebe, S., I.M. Rao, C. Cajiao, and M. Grajales. 2008. Selection for drought resistance in common bean also improves yield in phosphorus limited and favorable environments. *Crop Sci.* 48:582–592. doi:10.2135/cropsci2007.07.0404
- Beebe, S., I.M. Rao, M. Devi, and J. Polania. 2014. Common beans, biodiversity, and multiple stress: Challenges of drought resistance in tropical soils. *Crop Pasture Sci.* 65:667–675. doi:10.1071/CP13303
- Blum, A. 2005. Drought resistance, water-use efficiency, and yield potential are they compatible, dissonant, or mutually exclusive? *Aust. J. Agric. Res.* 56:1159–1168. doi:10.1071/AR05069
- Blum, A. 2009. Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. *Field Crops Res.* 112:119–123. doi:10.1016/j.fcr.2009.03.009
- Blum, A. 2015. Towards a conceptual ABA ideotype in plant breeding for water limited environments. *Funct. Plant Biol.* 42:502–513. doi:10.1071/FP14334
- Brito, G.G., N.D. Suassuna, V.N. Silva, V. Sofiatti, V. Diola, and C.L. Morello. 2014. Leaf level carbon isotope discrimination and its relationship with yield components as a tool for cotton phenotyping in unfavorable conditions. *Acta Sci., Agron.* 36:335–345. doi:10.4025/actasciagron.v36i3.17986
- Condon, A.G., R.A. Richards, G.J. Rebetzke, and G.D. Farquhar. 2004. Breeding for high water-use efficiency. *J. Exp. Bot.* 55:2447–2460. doi:10.1093/jxb/erh277
- Davies, W.J., and J. Zhang. 1991. Root signals and the regulation of growth and development of plants in drying soil. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 42:55–76. doi:10.1146/annurev.pp.42.060191.000415

- del Pozo, A., A. Yáñez, I.A. Matus, G. Tapia, D. Castillo, L. Sanchez-Jardón, and J.L. Araus. 2016. Physiological traits associated with wheat yield potential and performance under water-stress in a Mediterranean environment. *Front. Plant Sci.* 7:967. doi:10.3389/fpls.2016.00987
- Diaz, L.M., J. Ricarte, E. Tovar, C. Cajiao, H. Teran, M. Grajales, et al. 2018. QTL analyses for tolerance to several abiotic stresses in a common bean (*Phaseolus vulgaris* L.) population. *PLoS One* 13:e0202342. doi:10.1371/journal.pone.0202342
- Easlon, H.M., K.S. Nemali, J.H. Richards, D.T. Hanson, T.E. Juenger, and J.K. McKay. 2014. The physiological basis for genetic variation in water use efficiency and carbon isotope composition in *Arabidopsis thaliana*. *Photosynth. Res.* 119:119–129. doi:10.1007/s1120-013-9891-5
- Ehleringer, J.R., S. Klassen, C. Clayton, D. Sherrill, M. Fuller-Holbrook, Q.A. Fu, and T.A. Cooper. 1991. Carbon isotope discrimination and transpiration efficiency in common bean. *Crop Sci.* 31:1611–1615. doi:10.2135/cropsci1991.0011183X03100060046x
- El-Sharkawy, M.A., J.H. Cock, and A.D.P. Hernández. 1985. Stomatal response to air humidity and its relation to stomatal density in a wide range of warm climate species. *Photosynth. Res.* 7:137–149. doi:10.1007/BF00037004
- Farquhar, G.D., J.R. Ehleringer, and K.T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 40:503–537. doi:10.1146/annurev.pp.40.060189.002443
- Farquhar, G.D., and T.D. Sharkey. 1982. Stomatal conductance and photosynthesis. *Annu. Rev. Plant Physiol.* 33:317–345. doi:10.1146/annurev.pp.33.060182.001533
- Fischer, R.A., D. Rees, K.D. Sayre, Z.-M. Lu, A.G. Condon, and A. Larque-Saavedra. 1998. Wheat yield progress is associated with higher stomatal conductance, higher photosynthetic rate and cooler canopies. *Crop Sci.* 38:1467–1475. doi:10.2135/cropsci1998.0011183X003800060011x
- Gilbert, M.E., M.A. Zwieniecki, and N.M. Holbrook. 2011. Independent variation in photosynthetic capacity and stomatal conductance leads to differences in intrinsic water use efficiency in 11 soybean genotypes before and during mild drought. *J. Exp. Bot.* 62:2875–2887. doi:10.1093/jxb/erq461
- Kalaydjieva, R., A. Matev, and Z. Zlatev. 2015. Influence of irrigation regime on the leaf area and leaf area index of French bean (*Phaseolus vulgaris* L.). *Emir. J. Food Agric.* 27:171–177. doi:10.9755/ejfa.v27i2.19271
- Kaler, A., S. Bazzar, A. Sanz-Saez, J.D. Ray, F.B. Fritsch, and L. Purcell. 2018. Carbon isotope ratio fractionation among plant tissues of soybean. *Plant Phenome J.* 1:180002. doi:10.2135/tppj2018.04.0002
- Kerstiens, G. 1996. Cuticular water permeability and its physiological significance. *J. Exp. Bot.* 47:1813–1832. doi:10.1093/jxb/47.12.1813
- Klaedtke, S.M., C. Cajiao, M. Grajales, J. Polania, G. Borrero, and A. Guerrero. 2012. Photosynthate remobilization capacity from drought-adapted common bean (*Phaseolus vulgaris* L.) lines can improve yield potential of interspecific populations within the secondary gene pool. *J. Plant Breed. Crop Sci.* 4:49–61.
- Koester, R.P., J.A. Skoneczka, T.R. Cary, B.W. Diers, and E.A. Ainsworth. 2014. Historical gains in soybean (*Glycine max* Merr.) seed yield are driven by linear increases in light interception, energy conversion, and partitioning efficiencies. *J. Exp. Bot.* 65:3311–3321. doi:10.1093/jxb/eru187
- Martin, B., C.G. Tauer, and R.K. Lin. 1999. Carbon isotope discrimination as a tool to improve water-use efficiency in tomato. *Crop Sci.* 39:1775–1783. doi:10.2135/cropsci1999.3961775x
- Medrano, H., M. Tomás, S. Martorell, J. Flexas, E. Hernández, J. Rosselló, et al. 2015. From leaf to whole-plant water use efficiency (WUE) in complex canopies: Limitations of leaf WUE as a selection target. *Crop J.* 3:220–228. doi:10.1016/j.cj.2015.04.002
- Merah, O., E. Deleens, I. Souyris, M. Nachit, and P. Monneveux. 2001. Stability of carbon isotope discrimination and grain yield in durum wheat. *Crop Sci.* 41:677–681. doi:10.2135/cropsci2001.413677x
- Munoz, P., J. Voltas, J.L. Araus, E. Igartua, and I. Romagosa. 1998. Changes over time in the adaptation of barley releases in north-eastern Spain. *Plant Breed.* 117:531–535. doi:10.1111/j.1439-0523.1998.tb02202.x
- Pantin, F., T. Simonneau, G. Rolland, M. Dauzat, and B. Muller. 2011. Control of leaf expansion: A developmental switch from metabolics to hydraulics. *Plant Physiol.* 156:803–815. doi:10.1104/pp.111.176289
- Passioura, J.B. 1977. Grain yield, harvest index, and water use of wheat. *J. Aust. Inst. Agric. Sci.* 43:117–120.
- Polanía, J., I.M. Rao, S. Beebe, and R. García. 2009. Desarrollo y distribución de raíces bajo estrés por sequía en frijol común (*Phaseolus vulgaris* L.) en un sistema de tubos con suelo. *Agron. Colomb.* 27:25–32.
- Polania, J., I.M. Rao, C. Cajiao, M. Grajales, M. Rivera, F. Velasquez, et al. 2017a. Shoot and root traits contribute to drought resistance in recombinant inbred lines of MD 23–24 × SEA 5 of common bean. *Front. Plant Sci.* 8:296. doi:10.3389/fpls.2017.00296
- Polania, J., I.M. Rao, C. Cajiao, M. Rivera, B. Raatz, and S. Beebe. 2016a. Physiological traits associated with drought resistance in Andean and Mesoamerican genotypes of common bean (*Phaseolus vulgaris* L.). *Euphytica* 210:17–29. doi:10.1007/s10681-016-1691-5
- Polania, J., C. Poschenrieder, I. Rao, and S. Beebe. 2017b. Root traits and their potential links to plant ideotypes to improve drought resistance in common bean. *Theor. Exp. Plant Physiol.* 29:143–154. doi:10.1007/s40626-017-0090-1
- Polania, J.A., C. Poschenrieder, S. Beebe, and I.M. Rao. 2016b. Effective use of water and increased dry matter partitioned to grain contribute to yield of common bean improved for drought resistance. *Front. Plant Sci.* 7:660. doi:10.3389/fpls.2016.00660
- Rao, I.M. 2014. Advances in improving adaptation of common bean and *Brachiaria* forage grasses to abiotic stresses in the tropics. In: M. Pessarakli, editor, *Handbook of plant and crop physiology*. CRC Press, Boca Raton, FL. 847–889.
- Rao, I.M., S.E. Beebe, J. Polania, M. Grajales, C. Cajiao, J. Ricarte, et al. 2017. Evidence for genotypic differences among elite lines of common bean in their ability to remobilize photosynthate to increase yield under drought. *J. Agric. Sci.* 155:857–875. doi:10.1017/S0021859616000915
- Rao, I.M., S. Beebe, J. Polania, J. Ricarte, C. Cajiao, and R. Garcia. 2013. Can therapy bean be a model for improvement of drought resistance in common bean? *Afr. Crop Sci. J.* 21:265–281.
- Richards, R.A. 2006. Physiological traits used in the breeding of new cultivars for water-scarce environments. *Agric. Water Manage.* 80:197–211. doi:10.1016/j.agwat.2005.07.013

- Rosales, M.A., E. Ocampo, R. Rodríguez-Valentín, Y. Olvera-Carrillo, J. Acosta-Gallegos, and A.A. Covarrubias. 2012. Physiological analysis of common bean (*Phaseolus vulgaris* L.) cultivars uncovers characteristics related to terminal drought resistance. *Plant Physiol. Biochem.* 56:24–34. doi:10.1016/j.plaphy.2012.04.007
- Rosales-Serna, R., J. Kohashi-Shibata, J.A. Acosta-Gallegos, C. Trejo-López, J. Ortiz-Cereceres, and J.D. Kelly. 2004. Biomass distribution, maturity acceleration and yield in drought-stressed common bean cultivars. *Field Crops Res.* 85:203–211. doi:10.1016/S0378-4290(03)00161-8
- Singh, S.P., R. Lepiz, J.A. Gutierrez, C. Urrea, A. Molina, and H. Teran. 1990. Yield testing for early generation populations of common bean. *Crop Sci.* 30:874–878. doi:10.2135/cropsci1990.0011183X003000040022x
- Suarez-Salazar, J.C., J.A. Polania, A.T. Contreras Bastidas, L. Rodriguez Suarez, S. Beebe, and I. Rao. 2019. Agronomical, phenological and physiological performance of common bean lines in the Amazon region of Colombia. *Theor. Exp. Plant Physiol.* 30:303–320. doi:10.1007/s40626-018-0125-2
- Tilman, D., C. Balzer, J. Hill, and B.L. Befort. 2011. Global food demand and the sustainable intensification of agriculture. *Proc. Natl. Acad. Sci. USA* 108:260–264.
- United Nations. 2017. World population prospects: The 2017 revision, Vol. I: Comprehensive tables (ST/ESA/SER.A/399). United Nations, New York.
- Vadez, V., and P. Ratnakumar. 2016. High Transpiration efficiency increases pod yield under intermittent drought in dry and hot atmospheric conditions but less so under wetter and cooler conditions in groundnut (*Arachis hypogea* (L.)). *Field Crops Res.* 193:16–23. doi:10.1016/j.fcr.2016.03.001
- Valin, H., R.D. Sands, D. van der Mensbrugghe, G.C. Nelson, H. Ahammad, E. Blanc, and B. Bodirsky. 2014. The future of food demand: Understanding differences in global economic models. *Agric. Econ.* 45:51–67. doi:10.1111/agec.12089
- White, J.W., J.A. Castillo, and J.R. Ehleringer. 1990. Associations between productivity, root growth and carbon isotope discrimination in *Phaseolus vulgaris* under water deficit. *Aust. J. Plant Physiol.* 17:189–198.
- White, J.W., J.A. Castillo, J.R. Ehleringer, J.A. Garcia-C, and S.P. Singh. 1994a. Relations of carbon isotope discrimination and other physiological traits to yield in common bean (*Phaseolus vulgaris*) under rainfed conditions. *J. Agric. Sci.* 122:275–284. doi:10.1017/S0021859600087463
- White, J.W., M. Ochoa, P.F. Ibarra, and S.P. Singh. 1994b. Inheritance of seed yield, maturity and seed weight of common bean (*Phaseolus vulgaris*) under semi-arid rainfed conditions. *J. Agric. Sci.* 122:265–273. doi:10.1017/S0021859600087451
- Zhou, B., A. Sanz-Saez, A. Elazab, T. Shen, R. Sanchez-Bragado, J. Bort, et al. 2014. Physiological traits contributed to the recent increase in yield potential of winter wheat from Henan Province, China. *J. Integr. Plant Biol.* 56:492–504. doi:10.1111/jipb.12148