





RESEARCH ARTICLE

Variability in physiological responses of Venezuelan cacao to drought

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Abstract

In order to assess the response of cocoa trees to drought, changes in water status, gas exchange, leaf carbon isotopic ratio ($\delta^{13}\text{C}$), photochemical activity, and leaf N and chlorophyll content during the rainy and dry season were measured in 31 Venezuelan cocoa clones (17 Trinitarios, 6 Criollos, and 8 Modern Criollos) grown in a common garden. Drought caused a 40% decrease in water potential (ψ) in all but the Modern Criollos, and a reduction in net photosynthetic rate (A) and stomatal conductance (g_s) without an increase in instantaneous water use efficiency (WUE) in 93% of clones, and an increase in $\delta^{13}\text{C}$ (long-term WUE) in 74% of clones; these responses suggest differences in tolerance to drought among clones. A positive correlation between A and both g_s and leaf N content was found for all genotypes. Leaf N content, chlorophyll content, and photochemical activity were reduced during drought, suggesting that metabolism was also inhibited. The best performance during drought was shown by Modern Criollos with the highest WUE, while five Trinitario clones seemed to be less sensitive to drought, since neither chlorophyll, N, total soluble protein concentration, nor g_s changed with drought, indicating that those Trinitario clones, with lower A , have a conservative water use. Modern Criollos showed no reductions in either ψ or g_s ; A remained unchanged, as did WUE, which was the highest, suggesting that these clones would be more successful in environments with low water availability. Our results indicate large variation in physiological response to drought over a range of parameters, suggesting possible differences in tolerance among clones.

Keywords: Cocoa; Gas exchange; Carbon isotopic ratio; Drought; Specific leaf area; Water use efficiency

Introduction

Theobroma cacao L. (hereafter cocoa) is considered one of the most important perennial crops, with an estimated world output of 4.65 million tons in 2017–2018 (ICCO, 2019). Most smallholders in tropical developing countries cultivate it using rain-fed agroforestry (Carr and Lockwood, 2011). Yet, very often, their production is limited by water deficit as well as nutrients such as N (Balasimha *et al.*, 1991; dos Santos *et al.*, 2016). Amelioration in cocoa production should therefore consist of selecting clones able to cope with dry season (DS) without competing for water or nutrients.

Drought affects establishment and yield of cocoa (Bae *et al.*, 2008; dos Santos *et al.*, 2016), specially during the juvenile stage (Ayegboyin and Akinrinde, 2016; De Almeida *et al.*, 2016; García and Moreno, 2016). In adult trees, drought affected differently the water relations and photosynthetic responses of cocoa clones (Ávila-Lovera *et al.* 2016; Araque *et al.*, 2012; De Almeida *et al.*, 2016). Global climate change scenarios for South America predict lower rainfall averages during the DS (Gornall *et al.*, 2010). Cocoa plants submitted to drought showed different

responses which depended on the plant organ evaluated, that is, reduction of leaf area, and increase of root system and stem biomass strategic mechanisms for the survival of cocoa progenies under drought (dos Santos *et al.*, 2016). Therefore, it is essential to know the physiological responses of different cocoa clones to prolonged dry periods.

Decreases in yield caused by low water availability may largely be associated with a decline in net photosynthetic rate (A), either by a reduction in stomatal conductance (g_s) or by diminished metabolic processes (Lawlor and Tezara, 2009). In cocoa, low A was related to lower efficiency of Photosystem II (PSII) in the capture of excitation energy, as well as lower electron transport rate, J (Ávila-Lovera *et al.*, 2016; Tezara *et al.*, 2016). The adjustment of physiological processes, especially photochemistry and g_s feedbacks on A , and instantaneous water use efficiency ($WUE = A/E$, where E = transpiration rate) and yield in response to drought, which can provide functional details of growth and development in this tropical crop tree, are still not well understood (Daymond *et al.*, 2011).

In C_3 plants, the carbon isotopic ratio ($\delta^{13}C$) of leaf biomass gives a better indication of intercellular CO_2 concentration (C_i) over a longer time interval than instantaneous gas exchange measurements (Farquhar *et al.*, 1989). The value of C_i in itself is a reflection of g_s relative to A . As such, $\delta^{13}C$ provides an integrated or long-term WUE during the time when the carbon in leaf tissues was assimilated (Farquhar *et al.*, 1989; Farquhar and Richards, 1984). Long-term WUE is an important trait associated with yield and adaptation to water deficit (Monti *et al.*, 2006; Rajabi *et al.*, 2008). The ability to recognize genotypes that combine high yield with high WUE is essential when breeding crops for drought-prone areas (Acosta-Rangel *et al.*, 2018; Dias *et al.*, 2007; dos Santos *et al.*, 2016). Although the importance of screening large numbers of crops under similar agricultural management has been recognized, many breeding programs fail due to inappropriate clone choice for a certain region as a consequence of inadequate knowledge of the potential of clones and their growth and productivity under different site conditions (Rajabi *et al.*, 2008). A target trait at the leaf level for the selection of drought-tolerant woody crops such as cocoa could be WUE.

Cocoa is commonly divided into three morpho-geographic groups (morphotypes) which differ in quality, vigor, and yield: Criollo, Forastero, and Trinitario. Motamayor *et al.* (2008) suggested a new classification of cocoa grouped into 10 genetic groups, which would better reflect the genetic diversity available for breeders, among which the Criollo cultivars have a low genetic diversity (Motamayor *et al.*, 2002). On the whole, cocoa shows significant genetic variability regarding morphological and physiological traits (Daymond *et al.*, 2002a, b). However, studies of genotypic differences in photosynthetic performance of cocoa are few (Ávila-Lovera *et al.*, 2016; Daymond *et al.*, 2011; De Almeida *et al.*, 2016). Despite a wide genotypic variability (Motamayor *et al.*, 2008), most of the studies on yield reductions under water deficit have been reported only for Trinitario and Forastero clones (Almeida and Valle, 2007; Daymond *et al.*, 2011; De Almeida *et al.*, 2016; García and Moreno, 2016; Joly and Hahn, 1989), while little information on Criollo cocoa is available (Araque *et al.*, 2012; Ávila-Lovera *et al.*, 2016; Tezara *et al.*, 2016).

In order to gain knowledge on the changes in physiological traits during seasonal drought, we evaluated water status, gas exchange, leaf $\delta^{13}C$, photochemical activity, and leaf N and chlorophyll content in 31 cocoa clones grown in a common garden during the rainy season (RS) and DS. Our main question was Can the possible differences in physiological performance and responses of Venezuelan cocoa clones to drought suggest differences in drought tolerance? We hypothesized that during the DS, WUE would increase differently among clones due to differential physiological plasticity.

Materials and Methods

Plant material and field site

The study was carried out in the field germplasm bank at Instituto Nacional de Investigaciones Agrícolas (INIA)-Miranda Padrón Experimental Station, Tapipa, Venezuela (10°13'46'' N,

Table 1. Cocoa clones used in this study. Clone code, name, morphotype, location of origin with coordinates, mean annual rainfall

Clone code	Clone name	Morphotype	Location of origin	Annual rainfall (mm)	
T1	SC 6	Trinitario*	Barlovento	2200 ± 48	
T2	SC10		10°13' N, 66°18' W		
T3	PAN 75				
T4	PRO 63				
T5	CONCP 164				
T6	ORI 1	Trinitario*	Eastern Venezuela	1452 ± 24	
T7	ORI 4		10°35' N, 63°08' W		
T8	ORI 5				
T9	ORI 7				
T10	ORI 10				
T11	ORI 12	Trinitario*	Margarita Island	815 ± 34	
T12	MCC 3				11°01' N, 63°53' W
T13	MCC 4				
T14	MCC 5				
T15	MPR 1				
T16	MPR 11				
T17	MPR 14	Criollo†	Western Venezuela	1790 ± 56	
C1	OCC 2				08°50' N, 71°44' W
C2	OCC 4				
C3	OCC 6				
C4	OCC 11				
C5	OCC 14				
C6	OCC 17	Modern Criollo criollo‡	Central Coast	843 ± 58	
MC1	OC 60				10°31' N, 67°32' W
MC2	OC 63				
MC3	OC 66				
MC4	CHO 31				
MC5	CHO				
MC6	CHO 42				
MC7	CHUAO				
MC8	CHUAO 2				

*Trinitario are descendants of crosses between Criollo and Forastero.

†Pure Criollo, mostly with white seeds and high homozygosity percentage.

‡Modern Criollo are hybrids with many Criollo morphological traits.

66°18'39'' W). The climate corresponds to a tropical rainforest with a mean annual precipitation of 2480 ± 120 mm and an air temperature of 25.8 ± 1.9 °C. The rainfall regime of the study area is characterized by an RS with 317 ± 7 mm mo^{-1} from June to December and a DS with 75 ± 24 mm mo^{-1} from January to April (a mean of 10 years). Measurements were made in September during RS and April at the end of the DS. Measurements were made on five-year-old 2-m-tall trees of 31 cocoa clones (17 Trinitarios, 6 Criollos, and 8 Modern Criollos, i.e. hybrids between Criollo and Forastero with many Criollo morphological traits; Motamayor *et al.*, 2002), which were selected from the germplasm bank on the basis of their high productivity, and were propagated on open-pollinated IMC 67 rootstock. Clones were collected from five different geographical regions of Venezuela (Table 1). From now on, clones will be referred to by the number assigned in Table 1, instead of their names. Trees were planted following the germplasm policy in a common garden divided into five 400 m² square plots. Trees grew to 6–8 per clone per plot. Cocoa trees grew without irrigation under the shade of 15-m-tall trees of *Erythrina poeppigiana* (bucare) and lateral shade provided by 2.5-m-tall banana plants in every row. Banana trees were 1.5 m apart from each other and cocoa trees 3 m from each other. Trees were fertilized twice a year with commercial fertilizer triple 15-15-15 N:P:K. Soils in the study site were composed of 13.2% sand, 65.2 loam, and 21.6% clay, pH 6.4, available N, P, and K contents of 1.8, 4–8, and 535 mg kg^{-1} , respectively, and a cation exchange capacity of 0.44 dS m^{-1} . No tree died during

the period of this study as a result of drought or, for that matter, due to any other damage, such as disease.

Microclimatic conditions

Photosynthetic photon flux density (PPFD) was measured above cocoa trees with an LI-185 quantum sensor meter (LI-COR Inc., Lincoln, NE, USA), air temperature with YSI 405 thermistors connected to a telethermometer (Yellow Springs, OH, USA), and relative humidity (RH) with a hair strand hygrometer (Abbeon model AB167B, Santa Barbara, CA, USA). Leaf water vapor pressure deficit (Δ_w) was calculated as the difference in absolute humidity between the leaf intercellular spaces and the atmosphere, where water vapor pressure in the leaf was calculated with values of leaf temperature in the Infrared Gas Analyzer (IRGA) leaf chamber when gas exchange was measured (see below). Each microclimatic parameter was measured every hour for three consecutive days during each season when the physiological measurements were made.

Physiological measurements

Six trees of each clone were sampled to a total of 186 trees per season. All physiological measurements were made on the third mature, fully expanded leaf belonging to a mature branch.

Water relations

Morning leaf xylem water potential (ψ) was measured in one leaf of three trees ($n = 3$) per clone at 0600–0800 h with a pressure chamber (PMS Instruments Inc., Corvallis, OR, USA). Soil water content (SWC) at 30 cm was determined gravimetrically as SWC = water content/dry mass (%) in four replicates per plot. Soil samples were placed in metal containers, weighed, dried at 100 °C for 72 h and re-weighed.

Leaf gas exchange

Measurements of A , g_s , E , and WUE were made in one leaf per six individuals of each clone ($n = 6$) with a portable IRGA mod. LCA 4 used in conjunction with an assimilation chamber provided with a halogen lamp unit (Analytical Development Co., Hoddesdon, UK). Measurements were randomly made between 0900 and 1100 h in all clones during three consecutive days each season at ambient CO_2 concentration $380 \pm 10 \mu\text{mol mol}^{-1}$, PPFD $400 \pm 10 \mu\text{mol (photon) m}^{-2} \text{s}^{-1}$, previously determined to be saturating for many clones (Baligar *et al.*, 2008; Bertolde *et al.*, 2012; De Almeida *et al.*, 2018; Tezara *et al.*, 2016), and leaf temperature 28 ± 1 °C.

Specific leaf area, leaf N content, and $\delta^{13}\text{C}$

Specific leaf area (SLA) was calculated as the ratio between leaf area and dry mass in six individuals per clone. Leaf area was allometrically determined by weighing 1 cm² of paper and paper replicas of the leaves. Leaves were oven-dried to constant mass at 70 °C and weighed using a digital balance. Leaf samples of four individuals per clone ($n = 4$) were ground and then analyzed for $\delta^{13}\text{C}$ and leaf N content at the University of Illinois at Chicago using an element analyzer (Costech, Valencia, CA, USA) coupled to a Delta+XL isotope ratio mass spectrometer (Finnigan, Bremen, Germany) operated in continuous flow and run against standards from the National Institute of Standards and Technology and laboratory standards to a precision of 0.05% for C.

Biochemical determinations

Leaf chlorophyll and total soluble protein (TSP) contents were determined in discs ($n = 6$) taken from the same leaf in which gas exchange was measured; discs were enclosed in sealed polythene bags, transported to the laboratory, and maintained in the refrigerator until determinations were done. Total chlorophyll content (Clt) was determined in extracts obtained by grinding discs in chilled 80% acetone after Bruinsma (1963); TSP was determined according to Bradford (1976) in the macerate obtained by grinding discs over ice and following the procedure for the extraction of rubisco described by Tezara *et al.* (2002).

Chlorophyll *a* fluorescence of PSII

Chlorophyll fluorescence was measured on light-adapted attached leaves under actinic light of $150 \pm 10 \mu\text{mol (photon) m}^{-2} \text{ s}^{-1}$ PPFD for 2 min; this low value of PPFD was used because trees growing under bucare trees received low PPFD. Measurements were made in six trees per cultivar with a Mini PAM fluorometer (Walz, Effeltrich, Germany). Relative quantum yield of PSII at steady-state photosynthesis was calculated as $\Phi_{\text{PSII}} = (F'_m - F_s) / F'_m$, where F_s and F'_m are fluorescence at steady-state photosynthesis and maximum fluorescence in the light, respectively. Non-photochemical quenching (NPQ) was calculated from measurements of fluorescence as $\text{NPQ} = (F_m - F'_m) / F_m$. Electron transport rate of PSII was estimated as $J = \Phi_{\text{PSII}} \times \text{PPFD} \times 0.84 \times 0.5$. Measurements were randomly made in all clones during three consecutive days each season.

Statistical analysis

Mean values of the measured parameters were tested for differences between seasons and among clones by two-way analysis of variance (ANOVA) using the statistical software R (v 2.15.0, R Foundation for Statistical Computing, 2012) and Agricolae version 1.2–3. When the ANOVA indicated a significant overall treatment effect, Tukey's honest significance test (Tukey's honestly significant difference (HSD), $p < 0.05$) was carried out to compare means between seasons within a given clone. Principal component analysis (PCA) was conducted using the PAST 3 (PALEontological Statistics) software.

Results

Microclimatic conditions and water relations

Values of PPFD between 0800 and 1600 h under full sunshine ranged from 200 to 1200 $\mu\text{mol (photon) m}^{-2} \text{ s}^{-1}$; maximum PPFD during the RS on bucare-shaded cocoa trees was approximately 30% that under full sunshine. Air temperature and RH varied diurnally from 26 ± 1 to 33 ± 2 °C, and from 90 ± 5 to $60 \pm 2\%$, respectively, showing no seasonal variation; maximum Δ_w , though, was lower during the RS than the DS (1.8 ± 0.3 and 3.2 ± 0.1 KPa, respectively).

SWC decreased from 36 ± 3 in the RS to $16 \pm 1\%$ during the DS. All the clones had similar ψ during the RS; a significant difference between seasons was found, with ψ decreasing on average 40% during the DS in 81% of clones, except for Modern Criollo clones, which showed similar ψ (-0.50 MPa) during both seasons ($p = 0.048$, Figure 1). From RS to DS, values of ψ ranged from -0.37 to -1.18 MPa (Trinitario clones) and from -0.38 to -0.90 MPa (Criollos).

Leaf gas exchange

Significant differences in leaf gas exchange variables occurred among clones (Figure 1). On average, a decrease of 47 and 51% in A ($p = 0.035$) and of 63 and 50% in g_s of Trinitario and Criollo clones ($p = 0.025$), respectively, were observed between the RS and the DS. In Modern Criollos, A did not change with drought. High variability in g_s was observed in Trinitario clones (T6–T11)

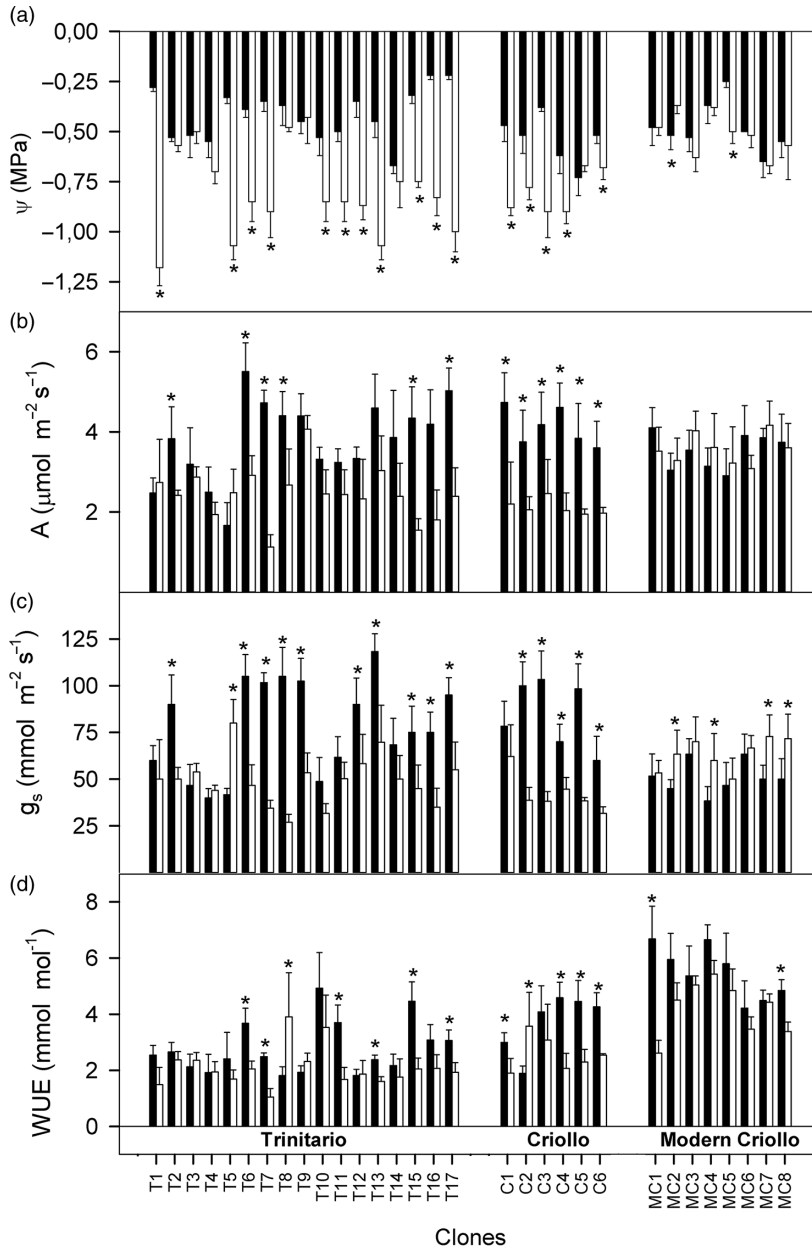


Figure 1. Seasonal changes in intact leaves of adult trees of cocoa clones grown in a common garden during the RS and the DS in (a) leaf water potential; (b) net photosynthetic rate; (c) stomatal conductance; and (d) WUE. Filled bars, RS; empty bars, DS. Values are mean \pm SE ($n = 6$) of different clones (indicated at bottom of panel (d)). An asterisk indicates significant differences due to season for each clone ($p < 0.05$, Tukey's HSD after a one-way ANOVA).

during both seasons and in Criollo clones (C1–C6) during the RS. The WUE was significantly reduced during the DS by 41% in Trinitario and 46% in Criollos ($p = 0.007$), while in Trinitarios T1–T5, with the lowest WUE ($\approx 2 \text{ mmol mol}^{-1}$), no change was observed between seasons. However, WUE in Modern Criollos was not only similar in both seasons but also significantly higher ($\approx 5 \text{ mmol mol}^{-1}$; $p = 0.012$) than in the other clones, the highest values

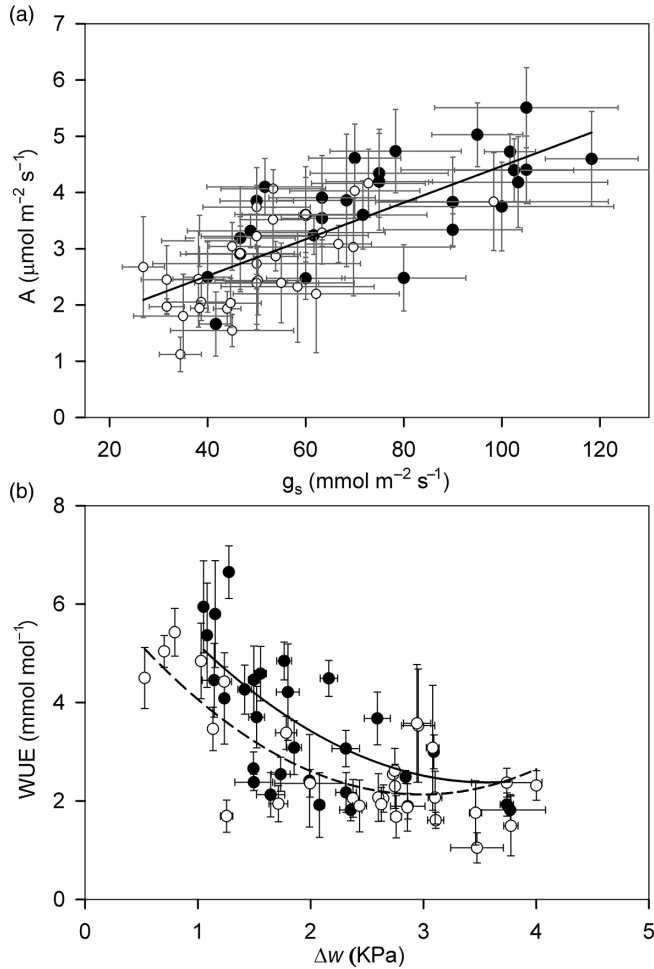


Figure 2. (a) The relationship between net photosynthetic rate and stomatal conductance of intact leaves of adult trees of 31 clones of *T. cacao* grown in a common garden during the RS (filled circle) and the DS (open circles). The linear regression including both seasons is represented by the solid line, $A = 0.03 \times g_s + 1.2$; $r^2 = 0.56$. (b) The response of WUE to leaf water vapor deficit of adult trees of *T. cacao* grown in a common garden during both seasons. Values are mean \pm SE ($n = 6$) of different clones. The non-linear regression fitted curves are included (solid for RS, broken for DS). The regression equations are $WUE = 0.4 \times \Delta_w^2 - 3.0 \times \Delta_w + 7.8$; $r^2 = 0.32$ for RS and $WUE = 0.5 \times \Delta_w^2 - 2.9 \times \Delta_w + 0.5$; $r^2 = 0.54$ for DS.

occurring in clones MC1–MC5. The interaction clone \times season for A and WUE was significant ($p = 0.049$ for A and $p = 0.01$ for WUE). During the DS, lower g_s was observed in Trinitario and Criollo clones, while Modern Criollo and Trinitario clones T1–T5 were not affected by drought.

The relationship between A and g_s was a straight line (Figure 2a, $p = 0.005$), changes in g_s explaining 56% of the variation in A . Values of A ranged from 1.7 to 5.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ associated to values of g_s from 40 to 120 $\text{mmol m}^{-2} \text{s}^{-1}$ during the RS and from 30 to 80 $\text{mmol m}^{-2} \text{s}^{-1}$ during the DS. The relationship between WUE and Δ_w was curvilinear in both seasons, the value of r^2 being slightly higher during the RS ($p < 0.05$, Figure 2b).

Leaf carbon isotopic composition as indicator of long-term WUE

Values of $\delta^{13}\text{C}$ (Figure 3) showed significant differences among clones ($p = 0.045$) and between seasons ($p = 0.039$). The difference between seasons in leaf $\delta^{13}\text{C}$ was higher in Criollos (approximately 1.62%). Trinitarios showed less variability between seasons (<1%) but had higher

variability among clones. Trinitario clones T2, T4, T9, T11, T15, T17, and T17 showed a large variation in $\delta^{13}\text{C}$ between seasons, while in the rest of them $\delta^{13}\text{C}$ had a small change. Increases in $\delta^{13}\text{C}$ were observed during the DS in most of clones independently of their site of origin ($-28.7 \pm 0.1\%$, compared with $-30 \pm 0.2\%$ in the RS). The highest $\delta^{13}\text{C}$ during the RS was observed in clone T8 and during the DS in C4 and T12. Values of $\delta^{13}\text{C}$ of all clones under study bore no correlation with either instantaneous WUE ($p = 0.08$) or intrinsic WUE, A/g_s ($p = 0.07$).

SLA and biochemical parameters

No significant seasonal changes in SLA were found in either most Trinitarios (T6–T17) or Criollos and Modern Criollos. However, variability in SLA was evident among clones from Barlovento (T1–T5) and between seasons, with the highest values in T2 and T3 ($222 \text{ cm}^2 \text{ g}^{-1}$) and the lowest in C2 ($114 \text{ cm}^2 \text{ g}^{-1}$). The interaction clone \times season for SLA was significant ($p = 0.000$; data not shown).

A positive correlation between A and area-based N content was found ($A = 1.07 \times N + 0.11$; $r^2 = 0.45$; $p = 0.047$; data not shown). No differences in TSP were found among clones but, during the DS, an increase was observed in seven Trinitarios, one Modern Criollo, and three Criollos (Figure 3). Leaf N and TSP contents were similar among clones, but leaf N content varied considerably between seasons in most clones ($p = 0.007$; Figure 3). On average, a reduction of 35% in leaf N content was observed during the DS. No significant interaction clone \times season was found for leaf N or TSP.

Chlorophyll a fluorescence parameters and chlorophyll content

In most clones, J was lower than $90 \mu\text{mol e}^- \text{ m}^{-2} \text{ s}^{-1}$, except for Trinitario clones T8 and T9; J was significantly higher during the RS in Trinitario from Eastern Venezuela and Margarita, with the exception of clone T8 ($p = 0.049$, Figure 4). No seasonally changes in J were found in Criollos (C2, C4, and C5) and Modern Criollos (MC2, MC3, MC4, and MC5), but in the rest of clones, differences were significant ($p = 0.005$) and there was interaction clone \times season ($p = 0.003$). During the DS, NPQ increased in Trinitario and Modern Criollos clones, except for T4, T14, T17, and MC1, no changes being observed in Criollo clones ($p = 0.92$, Figure 4). A significant reduction of 29% in chlorophyll content was observed during the DS, with the exception of T2, T1, T3, T11, MC2, MC4, and MC5 ($p = 0.048$, Figure 4), and there was interaction clone \times season ($p = 0.000$).

Principal component analysis

We used PCA to examine the level of coordination among selected traits across seasons and clones (Figure 5). PCA identified two primary axes of variation (principal components) that explained 54.1% of total variation. The first axis (PC1), which explained 31.2% of total variation, was positively associated with g_s , E , ΔW , PPF, J , and NPQ and negatively associated with WUE and Φ_{PSII} . The second axis (PC2), which explained 22.9% of total variation, was positive associated with ψ , A , WUE, and Clt and negatively associated with $\delta^{13}\text{C}$, ΔW , Φ_{PSII} , and TSP. The PCA fairly clearly showed separation of clones according to season.

Discussion

Inter-clone variations: genotype or seasonal rainfall?

The results of our study show a great genotypic variation on physiological traits such as water status, photosynthesis, WUE, and N content among cocoa clones in response to drought, which suggests differences in cocoa tolerance to drought. Modern Criollo, Criollo and some Trinitario clones had high WUE during both seasons. The strategies of optimization of water loss and CO_2

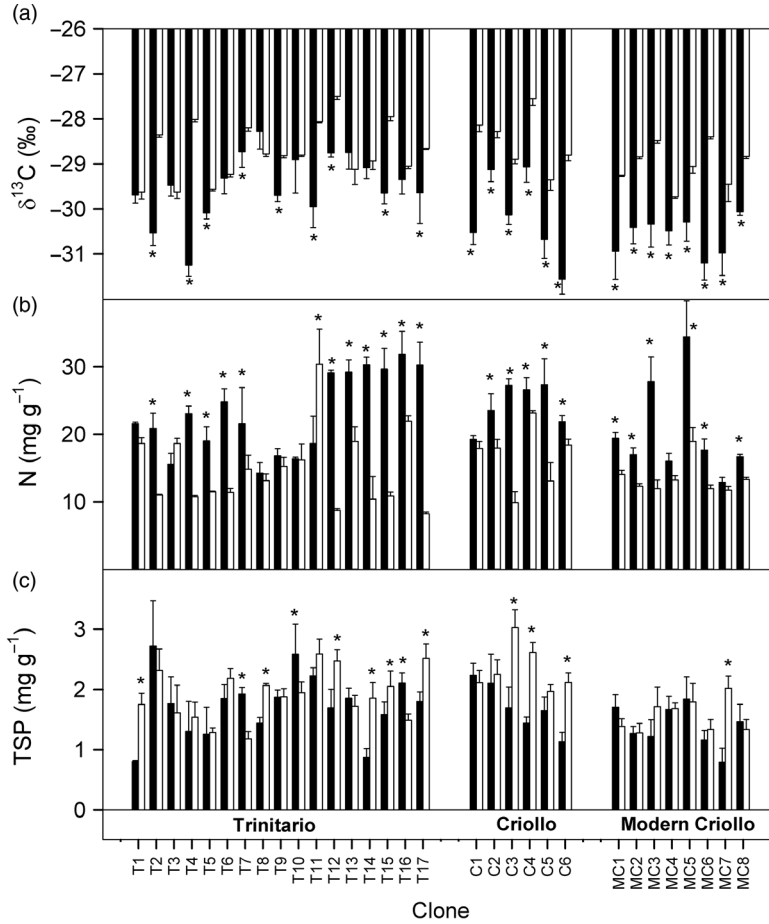


Figure 3. Seasonal changes in intact leaves of adult trees of cocoa clones grown in a common garden in (a) leaf carbon isotope ratio; (b) leaf nitrogen content; and (c) TSP. Filled bars, RS; empty bars, DS. Values are mean \pm SE ($n = 4$ for $\delta^{13}\text{C}$ and leaf N, and 6 for TSP) of different clones (indicated at bottom of panel (c)). An asterisk indicates significant differences due to season for each clone ($p < 0.05$, Tukey’s HSD after a one-way ANOVA).

assimilation were different among clones; some Trinitarios, for example, had higher g_s during the RS, while Modern Criollos and Trinitarios from Barlovento showed stomatal control under both seasons, causing differential patterns of $^{13}\text{CO}_2$ enrichment in the leaf. Our hypothesis that during drought, WUE would increase differently among clones due to great physiological plasticity was validated, since instantaneous WUE and $\delta^{13}\text{C}$ were significantly different in all clones despite trees growing under similar climatic, soil, and agronomic conditions, with $\delta^{13}\text{C}$ being values higher during the DS than the RS, suggesting a higher long-term WUE.

The ψ was poorly correlated with changes in A and g_s due to drought, whereas Δ_w had a more marked influence on such changes, especially in Modern Criollo. Our results suggest that in cocoa, drought causes decreases in A due to stomatal closure as well as metabolic inhibition, given the observed reductions in N, chlorophyll content, and electron transport rate.

Water relations

The lower precipitation during the DS, which halved SWC, caused a reduction of ψ in most of the clones under study. Similarly, a reduction of ψ was observed in Criollo trees (Araque *et al.*, 2012;

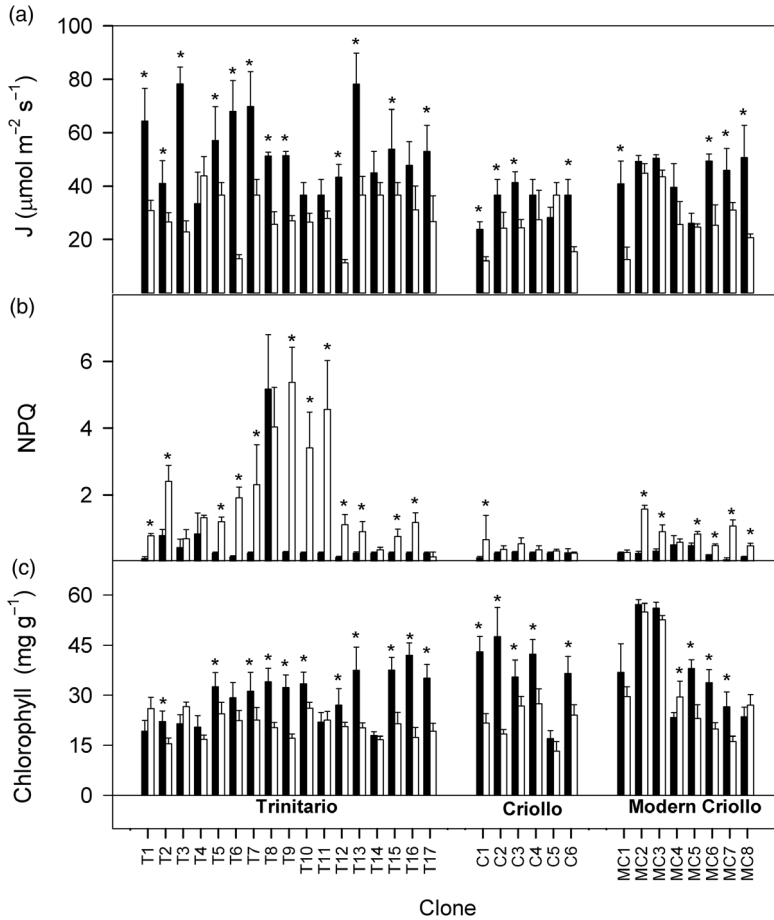


Figure 4. Seasonal changes in intact leaves of adult trees of cocoa clones grown in a common garden in (a) electron transport rate; (b) NPQ; and (c) total chlorophyll concentration. Filled bars, RS; empty bars, DS. Values are mean \pm SE ($n = 6$) of different clones (indicated at bottom of panel c). An asterisk indicates significant differences due to season for each clone ($p < 0.05$, Tukey's HSD after a one-way ANOVA).

Ávila-Lovera *et al.*, 2016; Rada *et al.*, 2005) and seedlings of Trinitario clones subjected to water deficit in different experiments in the greenhouse (Bae *et al.*, 2008; Joly and Hahn, 1989). In our study, in Modern Criollo and some Trinitario clones, ψ remained unchanged with drought. Cocoa clones more tolerant to drought generally differ physiologically, with mechanisms such as maximization of water uptake by deep, dense root systems, and/or minimization of water loss by stomatal closure (Almeida and Valle, 2007; dos Santos *et al.*, 2016). Genetic variation in the ability to tolerate drought in cocoa clones has been documented in only few occasions (Araque *et al.*, 2012; Ávila-Lovera *et al.*, 2016; Daymond *et al.*, 2011).

Leaf gas exchange

The low values of A observed in all clones were associated with low g_s . Similar ranges of A and g_s have been reported for Trinitario and Forastero clones of different ages grown in different conditions and environments worldwide (Acheampong *et al.*, 2013; Araque *et al.*, 2012; Ávila-Lovera *et al.*, 2016; Bae *et al.*, 2008; Baligar *et al.*, 2008; Daymond *et al.*, 2011; De Almeida *et al.*, 2018; Galyuon *et al.*, 1996; Joly and Hahn, 1989; Tezara *et al.*, 2016).

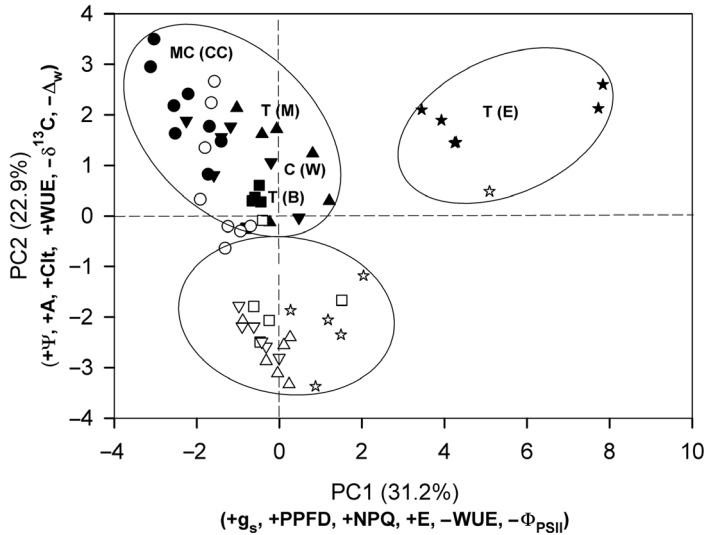


Figure 5. Bi-plot of PCA of the trade-offs among physiological traits evaluated of 31 clones of *T. cacao*: Trinitarios from Barlovento (B, squares), Trinitarios from eastern (E, star), Trinitarios from Margarita (M, triangles), western Criollos (W, inverted triangles), and Modern Criollos from Central Coast (CC, circles), grown in a common garden during the RS (filled symbols) and the DS (open symbols). Traits used for PCA analysis were xylem water potential (ψ), net photosynthetic rate (A), stomatal conductance (g_s), transpiration rate (E), instantaneous water use efficiency (WUE), SLA, total chlorophyll content (Clt), TSP, leaf nitrogen content (N), carbon isotopic ratio ($\delta^{13}C$), photosynthetic photon flux density (PPFD), leaf water vapor pressure deficit (Δ_w), relative quantum yield of PSII (Φ_{PSII}), non-photochemical quenching coefficient (NPQ), and electron transport rate (J).

We found variation in A and its response to drought in the clones studied, similarly to cocoa seedling in greenhouse conditions (Ayegboyin and Akinrinde, 2016; De Almeida *et al.*, 2016; García and Moreno, 2016) and cocoa trees in the field (Araque *et al.*, 2012; Ávila-Lovera *et al.*, 2016; De Almeida *et al.*, 2016). In most Trinitario and Criollo clones, A and g_s decreased during the DS, while in Modern Criollos, these variables were unaffected by drought. Decreases with drought of A in cocoa have been reported previously (Araque *et al.*, 2012; Acheampong *et al.*, 2013; Ávila-Lovera *et al.*, 2016; Ayegboyin and Akinrinde, 2016; Balasimha *et al.*, 1991), indicating sensitivity to water deficit, as reviewed by Almeida and Valle (2007) and Carr and Lockwood (2011).

There was a general trend toward stomatal closure with declining ψ during the DS, but this was not the cause for the decrease in WUE of Trinitario clones from Margarita and Eastern Venezuela and in Criollos because E was lower in these cases. Rather, diminished WUE was the result of lower A. Similar decreases in WUE with ψ have been reported in Forastero clones (Joly and Hahn, 1989). In contrast, in other Trinitarios, WUE increased with drought (Almeida and Valle, 2007; Baligar *et al.*, 2008). In some Criollo clones during the DS, WUE increased while ψ decreased (Rada *et al.*, 2005) or even remained unchanged (Araque *et al.*, 2012), similar to our findings in Trinitario clones (T1–T5). Clones responded differently to Δ_w , with most of them showing decreased WUE with increased Δ_w during both seasons; in Forastero clones measured under controlled conditions, E increased significantly with Δ_w (Baligar *et al.*, 2008).

The fact that g_s and A did not change seasonally in Modern Criollos probably allows these clones to be more productive during the DS than the other clones studied. In both seasons, the highest WUE was observed in the Modern Criollo clones, due to their lower g_s .

Carbon isotope ratio as indicator of long-term WUE

In both seasons, $\delta^{13}C$ was significantly different in all clones in spite of trees growing under similar conditions. Also, significant differences in $\delta^{13}C$ between seasons were observed, that is, values of

$\delta^{13}\text{C}$ were higher during the DS than the RS, suggesting that a higher long-term WUE was mostly due to stomatal closure (low g_s). The effects of physiological and biochemical factors on gas exchange are integrated by $\delta^{13}\text{C}$ in prolonged time scales and often related to WUE (Evans and von Caemmerer, 1996); higher $\delta^{13}\text{C}$ can be a consequence of either lower g_s or higher A , both leading to a higher long-term WUE. Values of $\delta^{13}\text{C}$ were lower in Modern and Criollo clones and Trinitarios from Barlovento during the RS, falling in the same range of values for a Trinitario and a Forastero clone under irrigation (Daymond *et al.*, 2011).

A poor relationship between $\delta^{13}\text{C}$ and instantaneous WUE and between $\delta^{13}\text{C}$ and A/g_s was obtained when data of all clones were pooled. A positive linear relationship between $\delta^{13}\text{C}$ and instantaneous WUE has been observed in fast-growing crop species such as wheat (Farquhar and Richards, 1984), barley (Hubick and Farquhar, 1989), cotton (Hubick and Gibson, 1993), beans (Monti *et al.*, 2006), and sugar beet (Rajabi *et al.*, 2008), and between A/g_s and instantaneous WUE in avocado (Acosta-Rangel *et al.*, 2018). In contrast, values of WUE in grapevines at different time scales did not follow the trend in values of $\delta^{13}\text{C}$ (Medrano *et al.*, 2015) and, in *Poa pratensis* (Ebdon *et al.*, 1998), environmental factors such as air temperature and Δ_w caused a negative relationship between $\delta^{13}\text{C}$ and WUE. These results indicate that WUE as evaluation tool among genotypes or treatments is not always advisable *a priori*.

Leaf characteristics and chlorophyll a fluorescence

The SLA across all clones was on average $143 \pm 9 \text{ cm}^2 \text{ g}^{-1}$, within the range reported in Forastero and Criollo clones in Eastern Venezuela (Tezara *et al.*, 2016) and lower than those reported in eight Forastero clones ($170\text{--}240 \text{ cm}^2 \text{ g}^{-1}$; Daymond *et al.*, 2011). The only clones that showed seasonal variation in SLA were Trinitarios (T1–T5), which also had the highest values, indicative of a more shaded provenance habitat. As a structural trait, SLA was less responsive to seasonal water availability than physiological traits such as A or $\delta^{13}\text{C}$.

Leaf N content was significantly higher in the RS than in the DS. Leaf N content ranged between 0.93 and 2.74 g m^{-2} , similar to values reported in eight Forastero clones (Daymond *et al.*, 2011) and lower than those reported in Criollo clones (Ávila-Lovera *et al.*, 2016; Tezara *et al.*, 2016). A positive correlation between A and area-based N content was found, as previously found in eight cocoa clones (Daymond *et al.*, 2011), indicating that A could have been affected during drought by both photochemical and biochemical limitations, since all processes of photosynthesis involve N.

Values of total chlorophyll content in the clones studied were consistent with those previously reported for Forastero and Criollo clones in the field (De Almeida *et al.*, 2018; Tezara *et al.*, 2016) but higher than previously reported for two Trinitario clones (Bertolde *et al.*, 2012) and half the chlorophyll content in Forastero clones (Daymond *et al.*, 2011). Drought considerably reduced chlorophyll content in Trinitario clones from Eastern Venezuela and Margarita and in Criollos, suggesting a lower capacity of absorption of light and electron transport in these clones during the DS.

Chlorophyll a fluorescence

The low values of J ($<90 \mu\text{mol e}^- \text{ m}^{-2} \text{ s}^{-1}$) in the present research are similar to those reported before in Criollo clones (Araque *et al.*, 2012; Ávila-Lovera *et al.*, 2016; De Almeida *et al.*, 2018; Tezara *et al.*, 2016) and lower than those found in other shade-tolerant crops such as tea (Mohotti *et al.*, 2000) and coffee (Martins *et al.*, 2014), in which J also saturates at low PPFD, suggesting that low A might have been due to low J . Reduction of J during drought is probably related to the lower chlorophyll content in this season, as was previously reported in two xerophytic species (Tezara *et al.*, 2005). Values of NPQ increased on some Trinitario clones and Modern Criollos, indicating a down-regulation of PSII activity.

Coordination of traits across clones and seasons

The two primary axes of variation in the PCA explained 54.2% of total variation. The PCA showed a clear separation in physiological performance during the RS and drought season in the cocoa clones studied, showing that some clones of Modern Criollos, Barlovento- and Eastern-Trinitarios were less affected by drought. The PC1 was positively associated with traits related to water use (g_s , E , Δ_w) and photochemical activity (PPFD, J , NPQ) and negatively associated with WUE and Φ_{PSII} , whereas the PC2 was positively associated with water status (ψ) and carbon economy (A , WUE) and negatively associated with $\delta^{13}C$.

Conclusions

The differences in physiological traits among the clones studied under the same microclimatic, soil, and agronomic conditions suggest high genotypic variation in Venezuelan cocoa. Variability in response to water deficiency over a range of variables suggests differences in tolerance to drought.

The ψ was poorly correlated with changes in A and g_s due to seasonal drought, whereas Δ_w had a more marked influence on such changes, especially in Modern Criollos. Our results suggest that in cocoa, drought causes decreases in A due to stomatal closure as well as metabolic inhibition, given the observed reductions in N content, chlorophyll content, and J .

All the Modern Criollos tested, Criollo C2 and C3, and Trinitario T8 and T10, with their better taste, flavor, and economic value, show potential for better performance during the DS. Complementary yield data are needed to evaluate which of these would be suitable as cultivars. The Trinitario clones from Barlovento, with lower WUE, seem to be less sensitive to adverse ambient factors (air temperature, humidity) than to intrinsic factors, since chlorophyll, N, and TSP content, along with g_s did not change with drought, indicating a conservative use of water and smaller capacity for carbon acquisition (lower A). During drought, Modern Criollos showed no reductions in either ψ , g_s or A . These clones showed the highest WUE in both seasons, suggesting that they would be more productive in environments with low water availability.

We conclude that clones MC1, MC2, C2, C3, T8, and T10 showed optimization of water use and potential tolerance to drought; therefore, these clones might be good alternatives for cocoa breeding programs in drier environments. However, in order to select clones tolerant to DS, data of pod production should be obtained to improve selection of better clones.

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