ECOPHYSIOLOGICAL TRAITS OF ADULT TREES OF CRIOLLO COCOA CULTIVARS (*THEOBROMA CACAO* L.) FROM A GERMPLASM BANK IN VENEZUELA

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SUMMARY

We studied physiological traits of 12 Criollo cocoa cultivars growing in a germplasm bank in the southern region of Maracaibo Lake Basin, during the rainy (RS) and dry seasons (DS) of 2007. A further evaluation of photosynthetic responses to changes in environmental parameters was done on three cultivars: Los Caños 001 (LCA001), Sur Porcelana 010 (SP010) and Escalante 001 (ESC001) in 2009 and 2010. Leaf water potential (Ψ_L) of most cultivars decreased during the DS of 2007, with the exception of ESC001. Maximum photosynthetic rate (A_{max}), stomatal conductance and water use efficiency varied among cultivars and seasons. The CO_2-saturated photosynthetic rate (A_{CO2sat}) was higher in LCA001 and ESC001 than in SP010, with no differences in carboxylation efficiency. Light curve responses of the three cultivars were similar. In all cultivars, no evidence of chronic photoinhibition was observed, since maximum quantum yield of photosystem II was high (0.77–0.81). We conclude that ESC001 has the best physiological performance (Ψ_L remained unchanged, highest A_{max}, A_{CO2sat} and photochemical activity), and it seems to be a promising cultivar for cocoa agroforestry systems in the southern region of Maracaibo.

INTRODUCTION

The cocoa tree (*Theobroma cacao* L., Malvaceae) (Alverson *et al.*, 1999) is considered one of the most important perennial crops in the world. It is economically important in many tropical countries, where it is cultivated by nearly 6 million farmers (Baligar *et al.*, 2008), with an estimated world output of 3.97 million tonnes in 2012/2013 (ICCO, 2013). Cocoa is a tropical woody species that typically grows in the understory of rain forests in areas of high annual rainfall (1,500–2,000 mm) (Bae *et al.*, 2008; Baligar *et al.*, 2008), and it is considered a shade tolerant plant as it grows well in moderate shade, and young plants suffer less water and nutrient stress under this condition (Wood and Lass, 2001).

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Cocoa exhibits considerable genetic variability regarding morphological and physiological traits (Daymond *et al.*, 2002a, b). However, studies of genotypic variation in photosynthetic traits in cocoa are limited (Daymond *et al.*, 2011). There are three types or morpho-geographic groups of cocoa known as 'Criollo', 'Forastero' and 'Trinitario', which differ in quality, vigor and yield (Cheesman, 1944). Recently, Motamayor *et al.* (2008) suggested a new classification of cocoa germplasm and grouped them into 10 genetic clusters, which would reflect the genetic diversity available for breeders in a better way than the previous classification. The Criollo cocoa identified within these 10 clusters has a low genetic variability (Motamayor *et al.*, 2002) and it is considered one of the best quality cocoa in the world (Elwers *et al.*, 2009). Venezuela has maintained Criollo cultivars in germplasm banks and recently, national programs are introducing some of these cultivars in cocoa farms in order to improve cocoa seed quality and amount of production.

Venezuelan cocoa contributes to less than 0.5% of the world cocoa production (ICCO, 2012). Furthermore, little is known about Venezuelan Criollo cocoa related to their physiological performance (water status, gas exchange and photochemical activity) or their responses to changing environmental conditions.

Climate change scenarios for Venezuela predicted less rainfall averages for DS (Gornall *et al.*, 2010). Therefore, it is important to select cultivars with higher tolerance to prolonged drought periods or climate regimes with less average precipitation. Water deficit is the main ecological factor that constrains photosynthesis in terrestrial ecosystems (McDowell *et al.*, 2008), limiting plant growth and survival (Chaves and Pereira, 1992; Chaves *et al.*, 2002). This is an important factor to consider for cultivation of cocoa since it requires a high amount of water ($>$ 1,500 mm rainfall a year), especially during the early juvenile stage (Carr and Lockwood, 2011).

The amount and distribution of rainfall represent important environmental factors that affect cocoa yield under field conditions (Alvim and Nair, 1986; Balasimha *et al.*, 1991). Cocoa plants are sensitive to prolonged periods of drought (Abo-Hamed *et al.*, 1983; Belsky and Siebert, 2003; Wood and Lass, 2001) and very little research has been directed towards the identification and development of drought tolerant Criollo cocoa cultivars. Only a few studies have examined differences in gas exchange among Forastero and Trinitario cocoa plants, and no significant differences were found in terms of photosynthetic rate (A), stomatal conductance (g_s), internal CO_2 concentration (C_i) and transpiration rate (E) between one Trinitario and two Forastero clones (Baligar *et al.*, 2008). However, A of two Trinitario clones responded differently to applied N in a greenhouse experiment, despite the existence of close genetic relatedness between them (Ribeiro *et al.*, 2008).

Some information for few cultivars from the western region in Venezuela is available (Araque *et al.*, 2012; Rada *et al.*, 2005; Tezara *et al.*, 2009). Comparative ecophysiological studies between different cultivars during RS and DS is a strategic way to select cocoa elite cultivars and for their management in agroforestry systems (Jaimez *et al.*, 2008, 2013).

Table 1. Name, acronym, pod weight, number of seeds per pod, seed colour and seed index (refers to the average dry mass per seed) of the studies cultivars.

Cultivar	Acronym	Description	Pod weight (g)	Seeds/pod	Seed colour	Seed index (g)
Umuquena	UMQ001	Hybrid with Criollo traits*	625.0 ± 126	26.5 ± 5.80	white	0.74 ± 0.01
Uvito	UVI003	Modern Porcelana[†]	476.2 ± 40.1	35.0 ± 2.3	white	1.20 ± 0.12
Escalante	ESC001	Ancestral Porcelana[†]	223.2 ± 73.2	23.1 ± 2.07	white	1.18 ± 0.05
Lobatera	LOB002 LOB022	Ancestral Andean Criollo*	432.4 ± 20.2	27.1 ± 1.22	white	1.42 ± 0.06
Los Caños	LCA001	Ancestral Criollo*	384.5 ± 0.00	27.4 ± 0.00	5RP[‡]	1.37 ± 0.00
San Simon	SSN004 SSN002	Modern Criollo*	563.9 ± 27.4	22.5 ± 1.89	white	1.60 ± 0.13
Chama	CHA010	Ancestral Porcelana Criollo*	519.0 ± 24.8	24.0 ± 1.05	white	1.50 ± 0.09
Caño Blanco	CBL005	Modern Porcelana Criollo[†]	485.1 ± 16.7	25.9 ± 1.63	white	1.39 ± 0.05
Novillero	NOV006	Ancestral Andean Criollo*	554.1 ± 49.0	24.1 ± 1.2	white	1.82 ± 0.08
Sur Porcelana	SP010	Sur del Lago Porcelana[†]	463.6 ± 49.0	23.6 ± 1.31	white	1.53 ± 0.10

*Collected from Táchira State.
[†]Collected from Zulia State.
[‡]According to Munsell table.

The principal aim of this study was to evaluate the ecophysiology of 12 Criollo cocoa cultivars grown under the same climatic and soil conditions in the southern region of Maracaibo Lake Basin, Venezuela, during RS and DS, to recognize traits that are associated with drought tolerance. The photosynthetic capacity of three Criollo cultivars was assessed to determine whether the commonly low measured values of g_s may explain low A due to high relative stomatal limitation (L_s).

MATERIALS AND METHODS

Plant material and field site

The cultivars were arranged in a single plot, each cultivar represented by 6 plants separated by 3.0 m between them. The plants were grown under the shade of banana (*Musa sp.*), *Erythrina fusca* and *Gliricidia sepium*. Most of the cultivars studied were ancestral Criollo cocoa cultivars from The Andes region (Table 1 shows the information available for the cultivars). The average pod weight of the cultivars is 460 g, and there are *c.* 25 seeds per pod with a seed index, average dry mass per seed, > 1 g (Table 1). All cultivars are considered Criollo, mostly with white seeds and high homozygosis percentage (> 90%; Marcano, 2007).

The study was conducted at the Criollo germplasm bank of Centro Socialista de Investigación y Desarrollo del Cacao (CESID-Cacao) (8°43′27″ N, 71°44′33″ W) in the southern region of Maracaibo Lake Basin, where the mean annual temperature is 27–28 °C and total rainfall is 1750–1880 mm. Adult trees (7 years old) from 12 Criollo cocoa cultivars were evaluated in March 2007 during the DS (rainfall 480 mm January to March, rain 2 days in February and the last 14 days of March), and in November
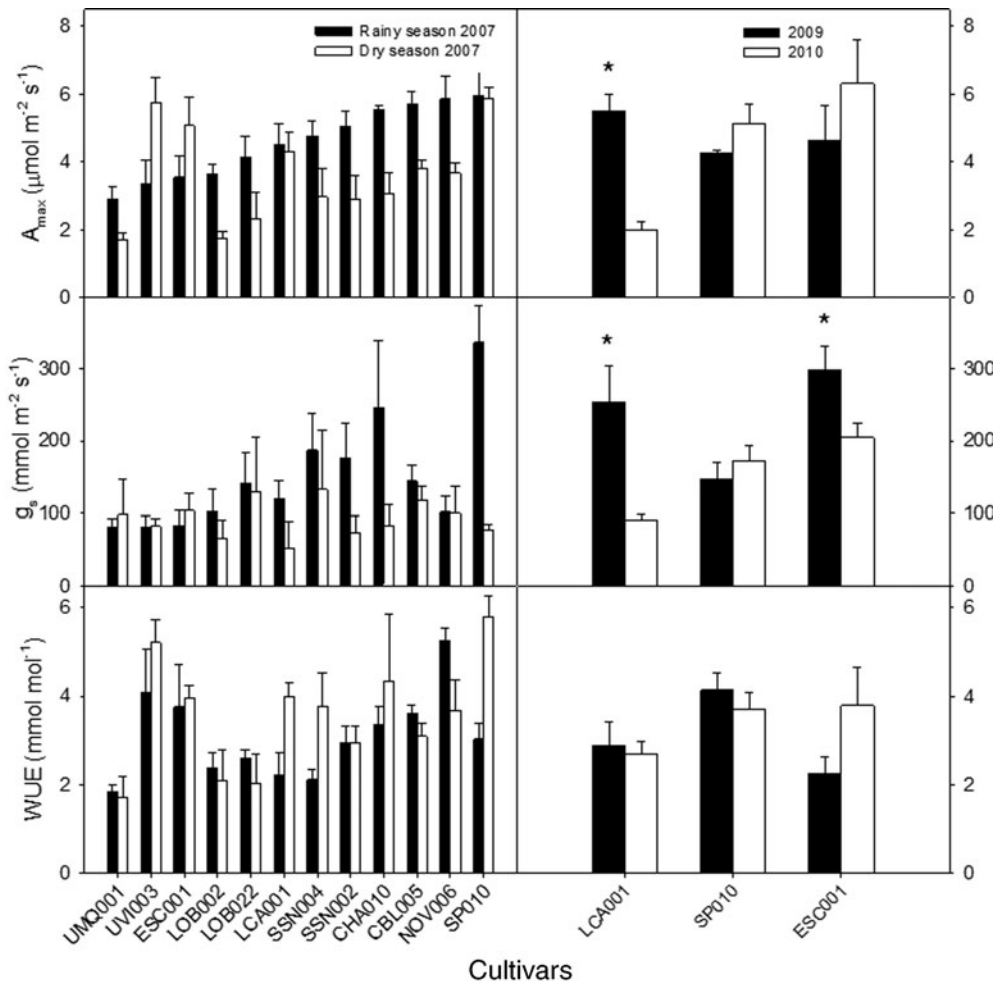
Figure 1. Measurements of instantaneous gas exchange: (a, d) photosynthetic rate (A), (b, e) stomatal conductance (g_s) and (c, f) WUE of 12 Criollo cocoa cultivars during RS and DS in 2007 and three selected cultivars measured in 2009 and 2010 from the southern region of Maracaibo Lake Basin. Values are mean ± SE ($n = 6$). Asterisk (*) in the right panel indicate significant difference between years for each cultivar ($p < 0.05$). Measurements were made at ambient (CO_2) (C_a) of 380 ± 10 μmol mol^{-1}, 21% O_2, PPFD of 400 ± 10 μmol m^{-2} s^{-1} and, a leaf temperature (T_L) of 28 ± 0.5 °C.

2007 during the RS (rainfall of 1900 mm between October and December, rain 25 out of 30 days in November). These cultivars grow under the same climatic and soil conditions, but were collected from different locations at the south western region of Venezuela: Táchira and Zulia states. After this first physiological evaluation (Figures 1 and 4), three cultivars: Los Caños 001 (LCA001), Sur Porcelana 010 (SP010) and Escalante 001 (ESC001), were selected to carry out a further evaluation during RS of 2009 and 2010 (rainfall of 1850 and 1980 mm between October to December, respectively).

Water relations

Leaf water potential (Ψ_L) was measured in 12 cultivars in early morning hours (~0600 h) in leaves of at least three individuals of each cultivar in both RS and DS in 2007, using a Scholander pressure chamber (PMS Instruments Inc., Corvallis, Oregon). Leaf relative water content (RWC) was determined in 2009 and 2010 for the three selected cultivars, according to the following equation: RWC = (FM–DM)/(TM–DM)*100, where, FM is fresh mass, DM is dry mass and TM is turgor mass. Turgor mass was calculated by rehydrating leaves in darkness overnight, which were weighted the following day.

GAS EXCHANGE

Instantaneous gas exchange measurements

Measurements of instantaneous maximum A (A_{max}), g_s and instantaneous water use efficiency (WUE = A_{max}/E) were done on fully expanded leaves in six individuals of each cultivar with a portable infrared gas analyser (CIRAS 2, PP Systems, Hitchin, UK) used in conjunction with an assimilation chamber (PLC, PP Systems, Hitchin, UK) and an attached LED light source. Measurements were made at ambient (CO_2) (C_a) of 380 ± 10 μmol mol^{-1}, 21% O_2, PPFD of 400 ± 10 μmol m^{-2} s^{-1} and leaf temperature (T_L) of 28 ± 0.5 °C. The A_{max} was measured between 8:30–10:00 h since previous measurements shown that photosynthesis is at maximum within these hours.

A/C_i and A/PPFD curves

Response curves of A to C_i (A/C_i curves) were done during RS in four individuals per cultivar (LCA001, SP010 and ESC001) by decreasing C_i from approximately 298 μmol mol^{-1} (at which A at C_a = 380 μmol mol^{-1} was initially measured) to 0 μmol mol^{-1} CO_2 and then progressively increasing it to 1200 μmol mol^{-1} CO_2. Measurements were done between 09:00–11:00 h at PPFD of 400 ± 10 μmol m^{-2} s^{-1}, 21% O_2, and T_L of 28 ± 0.5 °C.

The A/C_i curves were fitted to the empirical equation A = b + d*e^{k*Ci}, where b is C_i-saturated A (A_{CO2sat}) and (b+d) is diurnal respiration rate (R_d) (Tezara *et al.*, 1998). CE was calculated from the initial slope of the curve as k*d and the CO_2 compensation point (Γ) as Ln (–b/d)/k (Tezara *et al.*, 1998). Relative stomatal limitation (L_s) was calculated as L_s = 100 * (A_o–A)/A_o), where A_o is A at C_i = C_a (i.e. at infinite g_s) (Farquhar and Sharkey, 1982).

Response curves of A to PPFD (A/PPFD) were done during RS in four individuals per cultivar (LCA001, SP010 and ESC001) by decreasing PPFD from 400 μmol m^{-2} s^{-1} (at which A was initially measured) to 0 μmol m^{-2} s^{-1} and then progressively increasing it to 1400 μmol m^{-2} s^{-1} in eight steps, using the leaf microclimate control system of CIRAS 2. Measurements were done between 09:00–11:00 h at C_a of 380 ± 10 μmol mol^{-1}, 21% O_2 and T_L of 28 ± 0.5 °C.

The A/PPFD curves were fitted to the empirical equation A = b + d*e^{k*PPFD}, where b is PPFD-saturated A ($A_{PPFDsat}$) and (b+d) is dark respiration rate (R_D) (Tezara *et al.*,

1998). Apparent quantum yield (Φ_{CO2}) was calculated from the initial slope of the curve as k*d and light compensation point (LCP) as Ln (–b/d)/k (Tezara *et al.*, 1998).

<div align="center">STABLE ISOTOPE DETERMINATIONS AND NITROGEN CONTENT</div>

For 12 cultivars, leaf samples of adult individuals ($n = 4$) were ground and then analysed for carbon isotope composition ($\delta^{13}C$) and leaf N content at the University of Illinois-Chicago, using an elemental analyser (Costech, Valencia, California) coupled to a Delta + XL isotope ratio mass spectrometer (Finnigan, Bremen, Germany) operated in continuous flow and run against NIST and lab standards to a precision of 0.05‰ for C and 0.15‰ for N.

<div align="center">CHLOROPHYLL A FLUORESCENCE</div>

Chlorophyll *a* fluorescence was measured on attached dark-acclimated leaves ($n = 6$) of the three selected cultivars with a portable fluorometer (PAM 2100, Walz, Effeltrich, Germany) using the protocol described by Genty *et al.* (1989). Maximum quantum yield of PSII was measured *in situ* at predawn as $F_v/F_m = (F_m–F_o)/F_m$, where F_m and F_o are maximum and minimum fluorescence, respectively. Response curves of photochemistry parameters to PPFD were performed in four individuals per cultivar. Relative quantum yield of PSII (Φ_{PSII}) at steady state of A was calculated as $\Phi_{PSII} = (F'_m–F_s)/F'_m$, where F_s and F'_m are steady state and maximum fluorescence in light, respectively. Electron transport rate of PSII (J) was estimated as $J = \Phi_{PSII}*PPFD*a*0.5$, where *a* is the fraction of incident PPFD absorbed by the leaf (assumed as 0.84).

Photochemical (q_P) and non-photochemical (q_N) quenching coefficients were calculated from measurements of chlorophyll fluorescence as follows: $q_P = (F'_m–F_s)/(F'_m–F'_o)$ and $q_N = 1– (F'_m–F'_o)/(F_m–F_o)$, where F'_o is minimum fluorescence in light.

<div align="center">STATISTICAL ANALYSIS</div>

T-tests, one-way and two-way analyses of variance were performed to evaluate differences in the parameters measured between years, between cultivars, and between cultivars and seasons, respectively ($p < 0.05$). Fisher's least significant difference test was used as *post-hoc* analysis. SYSTAT 10 was used to run the analyses and Sigmaplot 11 to fit the curves.

<div align="center">RESULTS</div>

Water relations

Average Ψ_L of the 12 cultivars was significantly lower during DS compared to RS in 2007 (-0.41 ± 0.05 MPa and -0.21 ± 0.02, respectively, $p < 0.05$), as it was for LCA001 and SP010, while in ESC001 no differences were found between seasons (Table 2). There was no significant difference in RWC of these cultivars between 2009 and 2010, indicating high water availability during both sampling years (Table 2).

Table 2. Early morning leaf water potential (Ψ_L) and relative leaf water content (RWC) of three Criollo cocoa cultivars form the southern region of Maracaibo Lake Basin, during rainy and dry seasons in 2007 and rainy seasons in 2009 and 2010. Different letters between and within columns for Ψ_L indicate significant differences between cultivars and seasons/year, respectively ($p < 0.05$). (Means $\pm$ SE, $n = 3$).

Variable	Season/year	LCA001	SP010	ESC001
Ψ_L (MPa)	Rainy/2007	-0.20 ± 0.00^b	-0.29 ± 0.00^b	-0.23 ± 0.00^b
	Dry/2007	-0.51 ± 0.12^a	-0.54 ± 0.10^a	-0.18 ± 0.01^b
RWC (%)	Rainy/2009	80.2 ± 3.07^a	76.9 ± 5.84^a	77.0 ± 6.79^a
	Rainy/2010	81.8 ± 3.46^a	83.9 ± 0.41^a	85.0 ± 3.86^a

Gas exchange

There was a significant effect of season, cultivar and interaction season*cultivar in A_{max} and g_s values ($p < 0.05$), with A_{max} and g_s higher in RS than in DS for most cultivars (Figure 1). Maximum photosynthetic rate ranged between 2 and 6 μmol m^{-2} s^{-1}, with the highest values found in CHA010, CBL005 and NOV006 in RS, UVI003 and ESC001 in DS, and SP010 in both seasons (Figure 1a). The highest values of g_s where found in CHA010 and SP010 in RS, and in LOB022 and SSN004 in DS (Figure 1b). The highest A_{max} values did not fully correspond with the highest g_s values in all cultivars (Figures 1a–b), but the observed variation in A between these Criollo cacao cultivars can be partially explained by the variation in g_s ($r^2 = 0.47$, $p < 0.01$). WUE was different among cultivars ($p < 0.05$) and ranged between 2 and 6 mmol mol^{-1}, with the highest values found in NOV006 in RS and SP010 in DS (Figure 1c). There was no significant effect of season on WUE ($p > 0.05$).

The cultivars LCA001, SP010 and ESC001 were selected because of their high physiological performance: LCA001 had relatively high A_{max} and intermediate WUE in both seasons, SP010 had high A_{max} in both seasons and higher WUE in DS, and ESC001 had higher A_{max} in DS and WUE did not change between seasons.

Measurements of A_{max} and g_s in the three selected cultivars showed a different pattern between 2009 and 2010 (Figures 1d–f). The lowest A_{max} value was found in LCA001 in 2010 (2 μmol m^{-2} s^{-1}), while in the other cultivars and years the average was 5.2 μmol m^{-2} s^{-1} (Figure 1d). For all cultivars, g_s ranged between 90 and 300 mmol m^{-2} s^{-1}, with the highest values in LCA001 and ESC001 in 2009 (Figure 1e). Despite of differences in A_{max} and g_s, WUE was similar (3.3 mmol mol^{-1} on average) in all cultivars and years ($p > 0.05$; Figure 1f).

The A/C_i curves showed that LCA001 and ESC001 had the highest A_{CO2sat} (Figure 2), but CE, R_d, Γ and L_s were not different among the three cultivars (Table 3). The parameters of A/PPFD curves of the cultivars showed low $A_{PPFDsat}$ (in average 3.8 μmol m^{-2} s^{-1}), low values of saturating PPFD (400–500 μmol m^{-2} s^{-1}), R_D (-0.30 μmol m^{-2} s^{-1}) and LCP (11.1 μmol m^{-2} s^{-1}) (Figure 3), which are characteristic of shade plants. All cultivars showed no difference in the aforementioned parameters (Table 3).
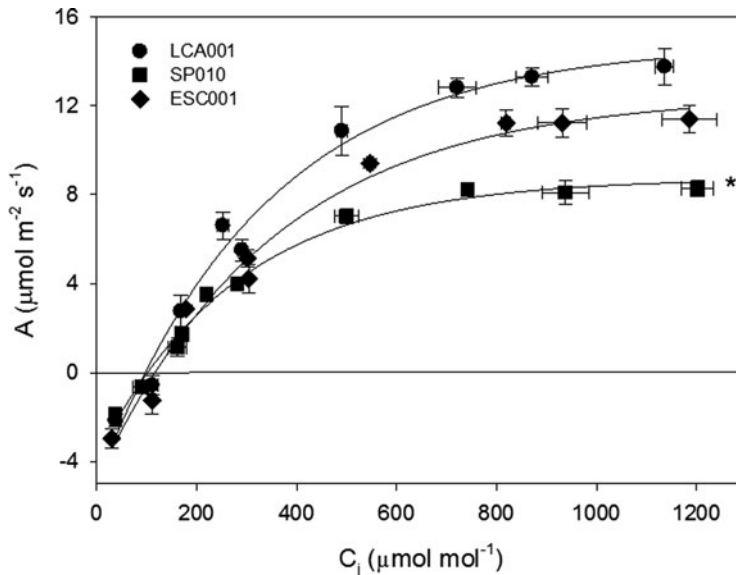
Figure 2. Response curves of photosynthetic rate (A) to intercellular CO_2 concentration (C_i) of three Criollo cocoa cultivars (● LCA001, ■ SP010, and ♦ ESC001) from the southern region of Maracaibo Lake Basin. Values are mean ± SE ($n = 4$). Asterisk (*) indicates that SP010 A_{CO2sat} was significant different from the others ($p < 0.05$). Measurements were made at PPFD of 400 ± 10 μmol m^{-2} s^{-1}, 21% O_2 and, T_L of 28 ± 0.5 °C.

Stable isotope determinations and nitrogen content

There was a significant effect of season, cultivar and interaction season*cultivar in $\delta^{13}C$ and N content for the 12 cultivars ($p < 0.05$) in 2007. Carbon isotope composition was found to be higher in RS than in DS, suggesting a higher integrated WUE during RS (Figure 4a). Nitrogen content was higher in RS than in DS in all evaluated cultivars (Figure 4b). There was a significant negative correlation between A and N content in RS, but not in DS ($p = 0.0163$ and $p = 0.2655$, respectively).

Chlorophyll a fluorescence

In all cultivars, F_v/F_m was higher in 2010 than 2009 ($p < 0.05$) with no differences among cultivars and no signs of photoinhibition in either year (Table 3). The response curves of J to PPFD showed that ESC001 had the highest maximum J (J_{max}) (Figure 5a). This is consistent with the higher Φ_{PSII} and q_P that this cultivar showed at high PPFD (Figures 5b and c), whereas q_N was higher in LCA001 than in the others cultivars at low PPFD (Figure 5d).

DISCUSSION

The response of physiological and photochemical traits of Criollo cocoa cultivars to seasonal drought was evaluated using adult plants grown in a germplasm bank without soil volume limitation, to identify traits that are associated with drought tolerance that could be used for cultivar screening-breeding programs. Our results showed variations

Table 3. Photosynthetic rate at saturating CO_2 (A_{CO2sat}), carboxylation efficiency (CE), diurnal respiration rate (R_d), CO_2 compensation point (Γ) and relative stomatal limitation (L_s) of LCA001, SP010 and ESC001. Photosynthetic rate at saturating PPFD ($A_{PPFDsat}$), apparent quantum yield (Φ_{CO2}), dark respiration rate (R_D) and light compensation point (LCP) of LCA001, SP010 and ESC001. Maximum quantum yield (F_v/F_m) in years 2009 and 2010. Different letters among columns indicate significant differences among cultivars ($p < 0.05$). (Means $\pm$ SE, $n = 4$).

| Variable		LCA001	SP010	ESC001
A/C_i curves				
A_{CO2sat} (μmol m^{-2} s^{-1})		14.6 $\pm$ 0.76[b]	8.81 $\pm$ 0.47[a]	12.70 $\pm$ 1.73[b]
CE (mol m^{-2} s^{-1})		0.065 $\pm$ 0.008[a]	0.051 $\pm$ 0.011[a]	0.052 $\pm$ 0.006[a]
R_d (μmol m^{-2} s^{-1})		$-$ 5.71 $\pm$ 1.37[a]	$-$ 4.52 $\pm$ 1.14[a]	$-$ 5.02 $\pm$ 1.39[a]
Γ (μmol mol^{-1})		101 $\pm$ 16.4[a]	105 $\pm$ 8.10[a]	117 $\pm$ 32.0[a]
L_s (%)		23.1 $\pm$ 4.22[a]	22.9 $\pm$ 5.09[a]	21.5 $\pm$ 9.78[a]
A/PPFD curves				
$A_{PPFDsat}$ (μmol m^{-2} s^{-1})		3.11 $\pm$ 0.00[a]	4.24 $\pm$ 0.57[a]	4.07 $\pm$ 0.40[a]
Φ_{CO2} (μmol μmol^{-1})		0.026 $\pm$ 0.000[a]	0.026 $\pm$ 0.003[a]	0.032 $\pm$ 0.003[a]
R_D (μmol m^{-2} s^{-1})		$-$ 0.17 $\pm$ 0.00[a]	$-$ 0.24 $\pm$ 0.06[a]	$-$ 0.50 $\pm$ 0.13[a]
LCP (μmol m^{-2} s^{-1})		6.52 $\pm$ 0.00[a]	10.5 $\pm$ 3.30[a]	16.1 $\pm$ 6.18[a]
Photochemistry activity				
F_v/F_m	2009	0.778 $\pm$ 0.006[a]	0.770 $\pm$ 0.012[a]	0.769 $\pm$ 0.012[a]
	2010	0.817 $\pm$ 0.006[b]	0.810 $\pm$ 0.007[b]	0.803 $\pm$ 0.003[b]
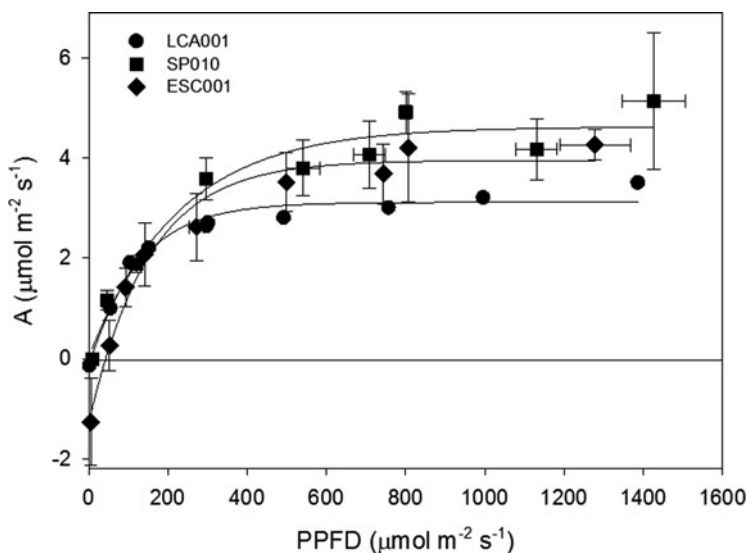
Figure 3. Response curves of photosynthetic rate (A) to photosynthetic photon flux density (PPFD) of three Criollo cocoa cultivars ($\bullet$ LCA001, $\blacksquare$ SP010, and $\blacklozenge$ ESC001) from the southern region of Maracaibo Lake Basin. Values are mean $\pm$ SE ($n = 4$). Measurements were made at C_a of 380 $\pm$ 10 μmol mol^{-1}, 21% O_2 and T_L of 28 $\pm$ 0.5 °C.

in physiological traits and differential responses to drought among cultivars; during the periods of low rainfall most cultivars showed a decrease in the leaf water status estimated by Ψ_L. The studied Criollo cultivars may be recommended for cultivation due to a relatively high integrated WUE estimated by δ^{13}C when comparing to Forastero and Trinitario cultivars from different ecosystems (Tezara *et al.*, 2009) and
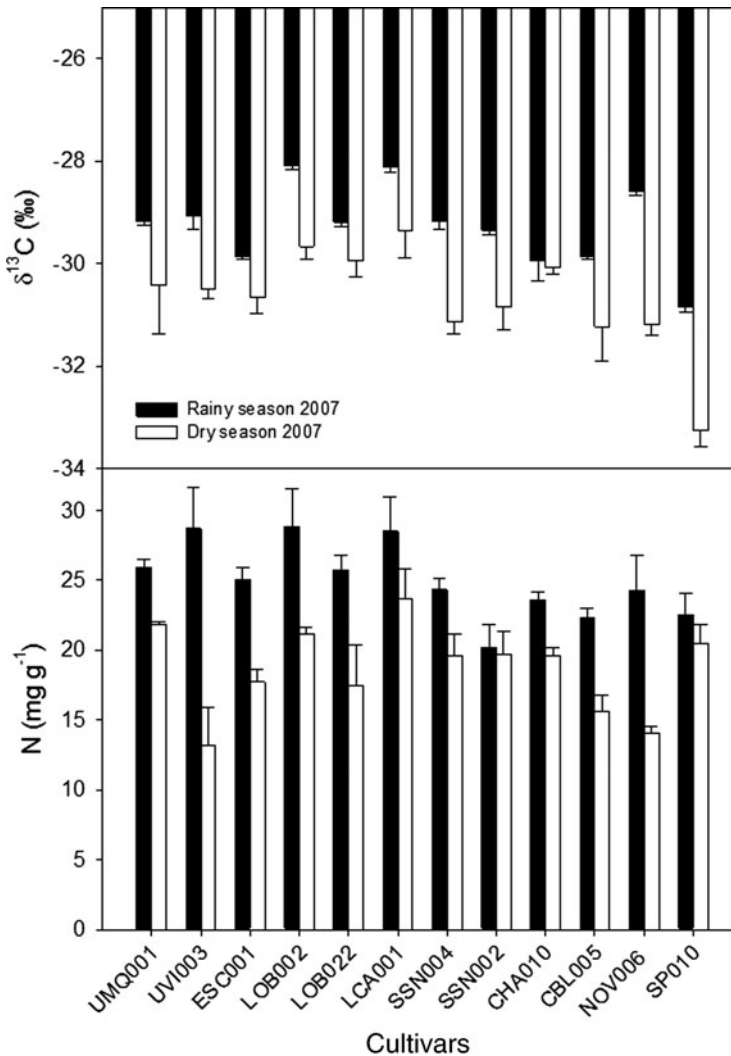
Figure 4. Isotopic composition (δ^{13}C) and nitrogen content (N) of 12 Criollo cocoa cultivars from the southern region of Maracaibo Lake Basin during rainy and dry seasons in 2007. Values are mean $\pm$ SE ($n = 4$).

other Criollo cultivars from agroforestry systems (Araque *et al.*, 2012) in Venezuela. Criollo cultivars showed low A_{max}, but the associated low g_s may not necessarily indicate that stomata explain the low rates of photosynthesis, a fact that is supported by low electron transport rate (≤ 80 μmol e$^-$ m^{-2} s^{-1}) and 23% of relative stomatal limitation in the three studied cultivars.

Water relations

On average, Ψ_L of the 12 cultivars was significantly higher in RS than in DS in 2007 except for ESC001, indicating that the water status for this cultivar was

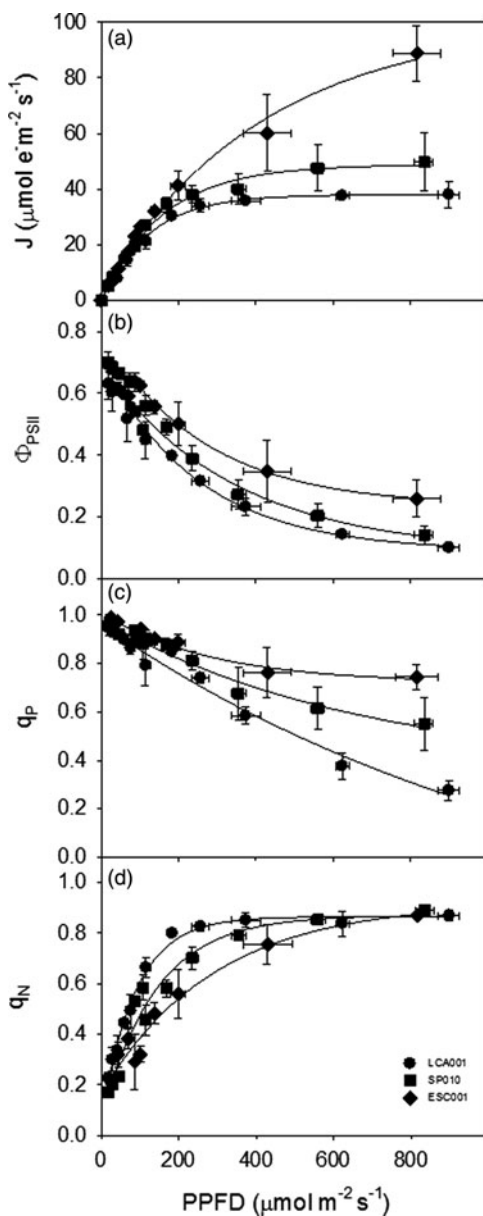Figure 5. Response curves of (a) electron transport rate (J), (b) relative quantum yield of photosystem II (Φ_{PSII}), (c) photochemical quenching coefficient (q_P) and (d) non-photochemical quenching coefficient (q_N) to photosynthetic photon flux density (PPFD) of three Criollo cocoa cultivars (● LCA001, ■ SP010, and ♦ ESC001) from the southern region of Maracaibo Lake Basin.

not affected by drought. During DS of 2007 rainfall was 25% of that in RS; this combined with high evaporative demand and high PPFD in the study area caused low water availability for most cultivars during DS, as it was observed before by Araque *et al.* (2012). Osmotic adjustment has been reported for some Forastero and

Criollo cocoa cultivars (Almeida and Valle, 2007; Araque *et al.*, 2012; Rada *et al.*, 2005) and it is usually associated with greater drought resistance (Almeida and Valle, 2007; Moser *et al.*, 2010). In our study case this hypothesis is not discarded. Leaf RWC values were around 80% with no difference between 2009 and 2010, which indicated no difference in water status of the plants in these two years. Lower Ψ_L with no changes in RWC could indicate osmotic adjustments through accumulation of solutes and/or changes in the cell wall module of elasticity in response to drought.

Gas exchange, water use efficiency and carbon isotope composition

The very low average A_{max} observed for all cultivars studied in 2007 (4.6 ± 0.31 and 3.6 ± 0.41 μmol m^{-2} s^{-1} in RS and DS, respectively) might be associated with low g_s (147.4 ± 20.3 and 86.2 ± 7.3 mmol m^{-2} s^{-1} in RS and DS, respectively). Similarly, A_{max} values have been found to range from 0.7 to 6.5 μmol m^{-2} s^{-1} with low g_s (20–150 mmol m^{-2} s^{-1}) in different studies (Almeida *et al.*, 2014; Baker and Hardwick, 1973, 1976; Daymond *et al.*, 2011; Joly and Hahn, 1989; Miyaji *et al.*, 1997a, b; Tezara *et al.*, 2009).

Different A_{max} and g_s responses to drought were found in 2007. In most Criollo cocoa cultivars, both A_{max} and g_s decreased during DS. Similar responses of A_{max} and g_s have been reported for some Trinitario, Forastero and Criollo cultivars of different ages and grown in different conditions and ecosystems (Acheampong *et al.*, 2013; Araque *et al.*, 2012; Baligar *et al.*, 2008; Daymond *et al.*, 2011; Galyuon *et al.*, 1996; Mohd Razi *et al.*, 1992; Joly and Hahn, 1989; Tezara *et al.*, 2009); all of them indicating that cocoa is sensitive to water deficit, as has been reported in recent reviews (Almeida and Valle, 2007; Carr and Lockwood, 2011).

During DS (2007) four cultivars showed an increase of instantaneous WUE, in six cultivars WUE was similar between seasons, and in two WUE decreased despite plants growing under similar environmental conditions (Figure 1). The reduction in g_s in some cultivars led to the increase in WUE during DS for some of these cultivars (e.g. CHA010 and SP010 more than 70%). It seems that these cultivars are less sensitive to drought in terms of A_{max} and WUE. These results have also been found in other Criollo cultivars from the southern region of Maracaibo Lake Basin (Rada *et al.*, 2005).

Stable carbon isotope composition revealed that, in all cultivars, long-termed integrated WUE was always higher in RS than in DS, although the opposite was expected as it is usually found: higher WUE during the period of water deficit (Farquhar and Richards, 1984). In this case, it seems that these cultivars came from natural populations that were selected and maintained due to their high WUE during RS, when most of growth and flowering occurs. Similar values of $\delta^{13}C$ have been reported for two clones of cocoa, ICS 1 and IMC 47 (Daymond *et al.*, 2011).

Nitrogen content

Leaf N content was significantly higher in RS than in DS ($p < 0.05$, Figure 4). All of the photochemical and biochemical process of photosynthesis involve nitrogen.

Indeed, nitrogenous compounds that provide the basis for photosynthesis include: proteins that catalyse the reactions of CO_2 fixation (by Rubisco) and the regeneration of the CO_2 acceptor (RuBP) (typically 16% nitrogen), chlorophyll (6% nitrogen), and thylakoids proteins (chlorophyll proteins, electron transport proteins and ATP-synthesizing enzyme) (Field and Mooney, 1986). In many species, growth under lower PPFD greatly increases the partitioning of nitrogen into chlorophyll and thylakoid proteins (Evans, 1989). At least eight cultivars in DS showed low A_{max} and N content suggesting that in those cultivars low A_{max} might be explained by this low N content. Total leaf N content in the Criollo cultivars studied ranged between 2 and 5 g m^{-2}, been those values higher than the reported in eight clones of cacao (Daymond *et al.*, 2011)

The fact that A is poor correlated with g_s in some cultivars during DS, but instead associated with the reduction of leaf N content ($r = 0.35$) suggests that biochemical factors could be regulating A. The typical positive correlation between photosynthesis and N content was not found in this study, perhaps due to the fact that A was area-based whereas the N content was weight-based. In general, the correlation coefficient between A_{max} and N content is higher for weight-based measurements ($r = 0.92$) than for area-based measurements ($r = 0.53$) (Field and Mooney, 1986). In contrast, a strong relationship between A and N was found in eight coca clones, where a high percentage of the variation in A was explained by the variation in N ($r^2 = 0.81$ and $p < 0.01$; Daymond *et al.*, 2011). In addition, some evergreen sclerophylls have high N per unit area but low A_{max}, as cocoa did, that could be possible due to the proportionally less nitrogen allocation to compounds functionally related to A_{max} and more allocation towards defence compounds (Field and Mooney, 1986).

Evaluation of parameters of A/C_i and A/PPFD curves, and chlorophyll fluorescence in three cultivars

The A/C_i curves showed that there was variation in A_{CO2sat} between cultivars, where the highest photosynthetic capacity was found in LCA001 and ESC001, although differences in CE were not found (Table 3). These results indicated that the three cultivars have similar Rubisco content and/or activity and for those with the highest A_{CO2sat}, probably a higher rate of RuBP regeneration (the highest J_{max} was also found in ESC001). Balasimha *et al.* (1991) have reported a linear relationship between A and C_i ($A = 0.015 Ci - 1.637$, $r^2 = 0.94$) in 16 Forastero cultivars (among drought tolerant and susceptible cultivars), with the highest value of A at a C_i of 230 μmol mol^{-1}. However, this might account just for the linear part of the A to C_i response curve. A quadratic response of A to C_i ($A = 5.55 + (-8.94)e^{(-0.01 Ci)}$, recalculated from Baligar *et al.*, 2008) was found in three Trinitario and Forastero cultivars from Perú, Ecuador and Brazil, and A_{CO2sat} was around 4 μmol m^{-2} s^{-1} (Baligar *et al.*, 2008), quite lower than the A_{CO2sat} found in this study. This indicated that under conditions of high CO_2 concentration, Criollo cultivars might have a higher A when comparing to Trinitario and Forastero cultivars. Currently, increases of C_a are known, and it is predicted to continue increasing over time (IPCC, 2014). This might suggest a benefit for Criollo cultivars in terms of photosynthetic capacity.

In spite of low g_s values, L_s was around 23% in the three cultivars evaluated in this study (Table 3). Similar results were found in Trinitario and Forastero cultivars, with L_s values around 22%, (recalculated data from Baligar *et al.*, 2008), and are similar to what is usually found in most C3 plants, *c.* 17% (Farquhar and Sharkey, 1982). The fact that cocoa showed low g_s does not necessarily indicate that stomata are regulating the rate of photosynthesis, a fact that is supported by the low L_s found in this study.

The parameters of A/PPFD curves of the Criollo cultivars showed adaptation to a shade regime, i.e. low values of $A_{PPFDsat}$, R_D and LCP. The saturating PPFD was relatively low, ranging from about 300 to 600 μmol m^{-2} s^{-1}, and similar to other cocoa cultivars (Almeida and Valle, 2007; Almeida *et al.*, 2014; Balasimha *et al.*, 1991; Baligar *et al.*, 2008; Joly and Hahn, 1989). Low yield of cocoa may be related to its low production of photoassimilates since A is low; however, variation in photosynthesis is not directly related to yield due to variation in canopy traits which might mask photosynthesis at the leaf level (Daymond *et al.*, 2002a, b). The apparent quantum yield observed in this studied was lower (0.028 μmol(CO$_2$) μmol(photon)$^{-1}$) than the reported for *T. cacao* (0.052 $\pm$ 0.016 μmol(CO$_2$) μmol(photon)$^{-1}$) and other species of the *Theobroma* genus (Almeida *et al.*, 2014), and slightly lower than the average reported in eight clones of cacao (0.033 $\pm$ 0.016 μmol(CO$_2$) μmol(photon)$^{-1}$; Daymond *et al.*, 2011) indicating a low use efficiency of light in these Criollo cocoa cultivars.

Despite of having the same water availability during 2009 and 2010 (according to RWC data), the three cultivars showed an increase in F_v/F_m in 2010 when comparing to 2009. This increase in F_v/F_m did not explain the decrease in A_{max} in LCA001; in this case, g_s explained it better. Low values of F_v/F_m ($\sim$ 0.7) have been reported in other Criollo cultivars from Maracaibo Lake Basin probably explained by a low P availability (Araque *et al.*, 2012). In our study, F_v/F_m did not drop below 0.75, suggesting absence of chronic photoinhibition.

Low values of J ($\leq$ 80 μmol e$^-$ m^{-2} s^{-1}) were found in these Criollo cocoa cultivars, which are lower than what has been found in other shade tolerant crops such as tea (Mohotti *et al.*, 2000) and coffee (Martins *et al.*, 2014), that also saturate at low PPFD; this suggests that low values of A_{max} found might be related to this low J. The response curves of photochemistry parameters to light in the three cultivars indicated that ESC001 had the best response to this resource, with the highest J, Φ_{PSII} and q_P at high PPFD values (Figure 5). Non-photochemical quenching was similar in all cultivars, suggesting that the mechanism of energy dissipation is similar in all of them, as it has been found in other Criollo cultivars in western Venezuela (Araque *et al.*, 2012).

In conclusion, our results indicated differences in physiological traits and differential responses to drought among the cultivars studied. All cultivars showed low A_{max}, but the associated low g_s may not necessarily indicate that stomata explain the low rates of photosynthesis, a fact that is supported by low values of J and L_s in the three cultivars selected. Maximum photosynthesis was poorly correlated with decreases in g_s in some cultivars during DS, but it was in accordance to low J which might cause reductions in RuBP regeneration and therefore low A values, suggesting that biochemical factors could be affecting photosynthesis more than stomatal. ESC001 seems to be a promising cultivar for cultivation in agroforestry systems in the southern

region of Maracaibo Lake Basin and other ecosystems with similar climatic conditions, due to its better response to drought (no decrease in Ψ_L during DS), best gas exchange performance (highest A_{max} and A_{CO2sat}) and best response of photochemical activity to light. However, pod yield information should support the selection of this cultivar. The other Criollo cultivars (LCA001 and SP010) can be recommended to be grown in areas with low rainfall periods occurring during the year due to their high long-termed integrated WUE (determined by $\delta^{13}C$).

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