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 CO₂-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).
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.

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

*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 gs may explain low A due to high relative stomatal limitation (Ls).

**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, c) 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 $(\text{CO}_2)$ ($C_a$) 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 ($\Psi_L$) 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: \[ RWC = \frac{(FM-DM)}{(TM-DM)} \times 100, \] 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 $A = b + d*e^{kC_i}$, 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 ($\Gamma$) 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 $A = b + d*e^{kPPFD}$, where b is PPFD-saturated A ($A_{PPFDsat}$) and (b+d) is dark respiration rate ($R_D$) (Tezara et al., 1998).
Apparent quantum yield ($\Phi_{\text{CO}_2}$) 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).

**STABLE ISOTOPE DETERMINATIONS AND NITROGEN CONTENT**

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.

**CHLOROPHYLL A FLUORESCENCE**

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 ($\Phi_{\text{PSII}}$) at steady state of A was calculated as $\Phi_{\text{PSII}} = (F'_m - F_s)/(F'_m - F'_o)$, where $F_s$ and $F'_m$ are steady state and maximum fluorescence in light, respectively. Electron transport rate of PSII ($J$) was estimated as $J = \Phi_{\text{PSII}} \times \text{PPFD} \times a \times 0.5$, 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: $q_P = (F'_m - F'_o)/(F'_m - F'_o)$ and $q_N = 1 - (F'_m - F'_o)/(F_m - F'_o)$, where $F'_o$ is minimum fluorescence in light.

**STATISTICAL ANALYSIS**

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.

**RESULTS**

**Water relations**

Average $\Psi_L$ of the 12 cultivars was significantly lower during DS compared to RS in 2007 ($-0.41 \pm 0.05$ MPa and $-0.21 \pm 0.02$, 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 ($\Psi_L$) and relative leaf water content (RWC) of three Criollo cocoa cultivars from 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 $\Psi_L$ indicate significant differences between cultivars and seasons/year, respectively ($p < 0.05$). (Means ± SE, $n = 3$).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Season/year</th>
<th>LCA001</th>
<th>SP010</th>
<th>ESC001</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Psi_L$ (MPa)</td>
<td>Rainy/2007</td>
<td>$-0.20 \pm 0.00^b$</td>
<td>$-0.29 \pm 0.00^b$</td>
<td>$-0.23 \pm 0.00^b$</td>
</tr>
<tr>
<td></td>
<td>Dry/2007</td>
<td>$-0.51 \pm 0.12^a$</td>
<td>$-0.54 \pm 0.10^a$</td>
<td>$-0.18 \pm 0.01^b$</td>
</tr>
<tr>
<td>RWC (%)</td>
<td>Rainy/2009</td>
<td>80.2 ± 3.07$^a$</td>
<td>76.9 ± 5.84$^a$</td>
<td>77.0 ± 6.79$^a$</td>
</tr>
<tr>
<td></td>
<td>Rainy/2010</td>
<td>81.8 ± 3.46$^a$</td>
<td>83.9 ± 0.41$^a$</td>
<td>85.0 ± 3.86$^a$</td>
</tr>
</tbody>
</table>

Gas exchange

There was a significant effect of season, cultivar and interaction season*cultivar in $A_{\text{max}}$ and $g_s$ values ($p < 0.05$), with $A_{\text{max}}$ and $g_s$ higher in RS than in DS for most cultivars (Figure 1). Maximum photosynthetic rate ranged between 2 and 6 $\mu$mol 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 $A_{\text{max}}$ 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 $A_{\text{max}}$ and intermediate WUE in both seasons, SP010 had high $A_{\text{max}}$ in both seasons and higher WUE in DS, and ESC001 had higher $A_{\text{max}}$ in DS and WUE did not change between seasons.

Measurements of $A_{\text{max}}$ and $g_s$ in the three selected cultivars showed a different pattern between 2009 and 2010 (Figures 1d–f). The lowest $A_{\text{max}}$ value was found in LCA001 in 2010 ($2 \mu$mol m$^{-2}$ s$^{-1}$), while in the other cultivars and years the average was 5.2 $\mu$mol 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 $A_{\text{max}}$ 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 $A_{\text{CO2sat}}$ (Figure 2), but CE, $R_d$, $\Gamma$ and $L_s$ were not different among the three cultivars (Table 3). The parameters of $A/PPFD$ curves of the cultivars showed low $A_{\text{PPFDsat}}$ (in average 3.8 $\mu$mol m$^{-2}$ s$^{-1}$), low values of saturating PPFD (400–500 $\mu$mol m$^{-2}$ s$^{-1}$), $R_d$ ($-0.30 \mu$mol m$^{-2}$ s$^{-1}$) and LCP (11.1 $\mu$mol 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 CO₂ concentration (Cᵢ) 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 ACO₂sat was significant different from the others (p < 0.05). Measurements were made at PPFD of 400 ± 10 μmol m⁻² s⁻¹, 21% O₂ and, Tₛ⁻ of 28 ± 0.5 °C.

Stable isotope determinations and nitrogen content
There was a significant effect of season, cultivar and interaction season*cultivar in δ¹³C 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, Fᵥ/Fₘ 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 PSI 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 CO₂ (A_{CO₂sat}), carboxylation efficiency (CE), diurnal respiration rate (R_d), CO₂ 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 ± SE, n = 4).

<table>
<thead>
<tr>
<th>Variable</th>
<th>LCA001</th>
<th>SP010</th>
<th>ESC001</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/CO₂ curves</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A_{CO₂sat} (μmol m⁻² s⁻¹)</td>
<td>14.6 ± 0.76b</td>
<td>8.81 ± 0.47a</td>
<td>12.70 ± 1.73b</td>
</tr>
<tr>
<td>CE (mol m⁻² s⁻¹)</td>
<td>0.065 ± 0.008a</td>
<td>0.051 ± 0.011a</td>
<td>0.052 ± 0.006a</td>
</tr>
<tr>
<td>R_d (μmol m⁻² s⁻¹)</td>
<td>−5.71 ± 1.37a</td>
<td>−4.52 ± 1.14a</td>
<td>−5.02 ± 1.39a</td>
</tr>
<tr>
<td>Γ (μmol mol⁻¹)</td>
<td>101 ± 16.4a</td>
<td>105 ± 8.10a</td>
<td>117 ± 32.0a</td>
</tr>
<tr>
<td>L_s (%)</td>
<td>23.1 ± 4.22a</td>
<td>22.9 ± 5.09a</td>
<td>21.5 ± 9.78a</td>
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<tr>
<td>A/PPFD curves</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>A_{PPFDsat} (μmol m⁻² s⁻¹)</td>
<td>3.11 ± 0.00a</td>
<td>4.24 ± 0.57a</td>
<td>4.07 ± 0.40a</td>
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<tr>
<td>Φ_{CO₂} (μmol μmol⁻¹)</td>
<td>0.026 ± 0.000a</td>
<td>0.026 ± 0.003a</td>
<td>0.032 ± 0.003a</td>
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<tr>
<td>R_D (μmol m⁻² s⁻¹)</td>
<td>−0.17 ± 0.00a</td>
<td>−0.24 ± 0.06a</td>
<td>−0.50 ± 0.13a</td>
</tr>
<tr>
<td>LCP (μmol m⁻² s⁻¹)</td>
<td>6.52 ± 0.00a</td>
<td>10.5 ± 3.30a</td>
<td>16.1 ± 6.18a</td>
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<td>Photochemistry activity</td>
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<tr>
<td>F_{v}/F_{m} 2009</td>
<td>0.778 ± 0.006a</td>
<td>0.770 ± 0.012a</td>
<td>0.769 ± 0.012a</td>
</tr>
<tr>
<td>2010</td>
<td>0.817 ± 0.006b</td>
<td>0.810 ± 0.007b</td>
<td>0.803 ± 0.003b</td>
</tr>
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</table>

Figure 3. Response curves of photosynthetic rate (A) to photosynthetic photon flux density (PPFD) of three Criollo cocoa cultivars (• LCA001, ■ SP010, and ♦ ESC001) from the southern region of Maracaibo Lake Basin. Values are mean ± SE (n = 4). Measurements were made at C_a of 380 ± 10 μmol mol⁻¹, 21% O₂ and T_L of 28 ± 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 δ¹³C when comparing to Forastero and Trinitario cultivars from different ecosystems (Tezara et al., 2009) and
other Criollo cultivars from agroforestry systems (Araque et al., 2012) in Venezuela. Criollo cultivars showed low $A_{\text{max}}$, 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 ($\leq 80 \mu\text{mol e}^{-}\text{m}^{-2}\text{s}^{-1}$) and 23% of relative stomatal limitation in the three studied cultivars.

**Water relations**

On average, $\Psi_L$ 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...
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 $\Psi_L$ 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 $A_{\text{max}}$ 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, $A_{\text{max}}$ 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 $A_{\text{max}}$ and $g_s$ responses to drought were found in 2007. In most Criollo cocoa cultivars, both $A_{\text{max}}$ and $g_s$ decreased during DS. Similar responses of $A_{\text{max}}$ 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 $A_{\text{max}}$ 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, IGS 1 and IMG 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 CO₂ fixation (by Rubisco) and the regeneration of the CO₂ 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 Aₘₐₓ and N content suggesting that in those cultivars low Aₘₐₓ 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 gₛ 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 Aₘₐₓ 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 (r² = 0.81 and p < 0.01; Daymond et al., 2011). In addition, some evergreen sclerophylls have high N per unit area but low Aₘₐₓ, as cocoa did, that could be possible due to the proportionally less nitrogen allocation to compounds functionally related to Aₘₐₓ and more allocation towards defence compounds (Field and Mooney, 1986).

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

The A/Cᵢ curves showed that there was variation in Aₜₒ₂ₛᵃᵗ 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 Aₜₒ₂ₛᵃᵗ, probably a higher rate of RuBP regeneration (the highest Jₘₐₓ was also found in ESC001). Balasimha et al. (1991) have reported a linear relationship between A and Cᵢ (A = 0.015Cᵢ − 1.637, r² = 0.94) in 16 Forastero cultivars (among drought tolerant and susceptible cultivars), with the highest value of A at a Cᵢ of 230 μmol mol⁻¹. However, this might account just for the linear part of the A to Cᵢ response curve. A quadratic response of A to Cᵢ (A = 5.55 + (−8.94)e(−0.01Cᵢ), recalculated from Baligar et al., 2008) was found in three Trinitario and Forastero cultivars from Perú, Ecuador and Brazil, and Aₜₒ₂ₛᵃᵗ was around 4 μmol m⁻² s⁻¹ (Baligar et al., 2008), quite lower than the Aₜₒ₂ₛᵃᵗ found in this study. This indicated that under conditions of high CO₂ concentration, Criollo cultivars might have a higher A when comparing to Trinitario and Forastero cultivars. Currently, increases of Cᵢ 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 $A_{PPFD_{sat}}$, $R_P$ and LCP. The saturating PPFD was relatively low, ranging from about 300 to 600 $\mu$mol 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 $\mu$mol(CO$_2$) $\mu$mol(photons)$^{-1}$) than the reported for $T$. cacao (0.052 ± 0.016 $\mu$mol(CO$_2$) $\mu$mol(photons)$^{-1}$) 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 ± 0.016 $\mu$mol(CO$_2$) $\mu$mol(photons)$^{-1}$; 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 $F_v/F_m$ (~ 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 $J$ ($\leq 80$ $\mu$mol 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 $J$, $\Phi_{PSII}$ 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 $\Psi_t$ during DS), best gas exchange performance (highest $A_{\text{max}}$ and $A_{\text{CO2sat}}$) 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-term integrated WUE (determined by $\delta^{13}$C).

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