LEAF GAS EXCHANGE OF NEW COCOA CLONES UNDER AN AGROFORESTRY SYSTEM IN ANTIOQUIA, COLOMBIA

INTERCAMBIO GASEOSO DE NUEVOS CLONES DE CACAO ESTABLECIDOS EN UN SISTEMA AGROFORESTAL EN ANTIOQUIA, COLOMBIA

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SUMMARY

Photosynthetic activity allows knowing the behavior of a species and its relationship with the environmental conditions where it develops, which can be modified by the components of an agroforestry system. In order to compare gas exchange variables in cocoa (*Theobroma cacao* L.) clones TCS01, TCS06, TCS13, TCS19 and CCN51 planted in an agroforestry system with abarco trees (*Cariniana pyriformis* Miers), net photosynthesis (A), stomatal conductance (g_s) and transpiration (E) were evaluated on a fully developed young cocoa leaf in six plants of each of the clones every hour from 08:00 to 17:00 hours (n = 5000) for five days. Areas under the curve (AUC) were estimated for each variable from the gas exchange data based on the sum of the fractionated trapezoidal areas. The AUC data were subjected to analysis of variance and the treatments were compared by the Tukey test ($P \leq 0.05$). Clone TCS06 presented the highest accumulated net photosynthesis (A) with a value of 137,300 μmol CO_2 m^{-2} day^{-1}, surpassing clones TCS19 and TCS01. Maximum values of A = 6.6 μmol CO_2 m^2 s^{-1} (TCS06), g_s = 0.12 mmol CO_2 m^{-2} s^{-1} (CCN51) and E = 4.52 mmol H_2O m^{-2} s^{-1} (CCN51) were found. Clones TCS06 and TCS01 stood out compared to TCS13, TCS19 and CCN51. Clone TCS06 presented the highest photosynthetic rate as a function of the daily integral of gas exchange compared to TCS19 and TCS13. CCN51 was the clone that required the highest photosynthetically active radiation to achieve maximum photosynthetic activity.

Index words: CO_2 assimilation rate, daily integral of gas exchange, photosynthesis, stomatal conductance, transpiration.

RESUMEN

La actividad fotosintética permite conocer el comportamiento de una especie y su relación con las condiciones ambientales donde se desarrolla, las cuales pueden ser modificadas por los componentes que integran un sistema agroforestal. Con el objetivo de comparar las variables de intercambio gaseoso en los clones de cacao (*Theobroma cacao*) TCS01, TCS06, TCS13, TCS19 y CCN51 plantados en un sistema agroforestal con árboles de abarco (*Cariniana pyriformis* Miers), se evaluó la fotosíntesis neta (A), la conductancia estomática (g_s) y la transpiración (E) en una hoja de cacao joven completamente desarrollada en seis plantas de cada uno de los clones, cada hora entre las 08:00 y 17:00 horas (n = 5000) por cinco días. Con los datos de intercambio gaseoso se estimaron las áreas bajo la curva (ABC) para cada variable con base en la sumatoria de las áreas trapezoidales fraccionadas. Los datos de las ABC fueron sometidos a análisis de varianza y los tratamientos comparados por la prueba de medias Tukey ($P \leq 0.05$). El clon TCS06 presentó la mayor fotosíntesis neta acumulada con un valor de 137.000 μmol CO_2 m^{-2} $día^{-1}$, superando a los clones TCS19 y TCS01. Se registraron valores máximos de A = 6.6 μmol CO_2 m^2 s^{-1} (TCS06), g_s = 0.12 mmol CO_2 m^{-2} s^{-1} (CCN51) y E = 4.52 mmol H_2O m^{-2} s^{-1} (CCN51). Los clones TC606 y TCS01 sobresalieron con respecto a TCS13, TCS19 y CCN51. El clon TCS06 presentó la mayor tasa fotosintética en función de la integral diaria del intercambio gaseoso en comparación con TCS19 y TCS13. El CCN51 fue el clon que requirió los mayores valores de radiación fotosintéticamente activa para lograr la máxima actividad fotosintética.

Palabras clave: Conductancia estomática, fotosíntesis, integral diaria del intercambio de gases, tasa de asimilación de CO_2, transpiración.

INTRODUCTION

Cocoa (*Theobroma cacao* L.) is a species native to the Amazon region of South America; its products are used worldwide for the chocolate, pharmaceutical and cosmetic industry (Wickramasuriya and Dunwell, 2018). The global market for cocoa beans is expected to grow at a compound annual rate of 7.3 % from 2019 to 2025 (Voora *et al*., 2019). World production in 2018 was led by Africa (71.1 %), followed by Latin America (16.1 %), Asia (11.9 %) and Oceania (1 %), with Ivory Coast being the main producing country (1,963,949 t). Colombia produced 52,743 tons in 2018, being the tenth world-producing country (FAO, 2020). Santander is the main producer state of Colombia (23,042 t per year), and it has a 38.06 % of Colombia planted area, followed by Antioquia (8.93 %), Arauca (8.32 %), Huila (7.97 %) and Tolima (7.58 %) (FEDECACAO, 2019).

Currently, there is a wide diversity of cocoa genotypes planted in Colombia. Clones TCS 01 (*Theobroma* Corpoica La Suiza), TCS 06, TCS 13 and TCS 19 are recommended by Agrosavia for the Mountain of Santander region (Agudelo *et al*., 2018; ICA, 2014). These clones are being

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evaluated for other regions of Colombia, such as the Mountain of Antioquia, due to their high yield (clones TCS 01 and TCS 19), disease resistance (clone TCS 06) and high quality of the grain. In Colombia, cocoa cultivation predominates in agroforestry systems with other perennial woody species (FEDECACAO, 2020). The shade generated in these systems modifies the environmental conditions and benefits the physiology of the crop providing greater longevity and stability in cocoa production (Jagoret *et al*., 2018; Niether *et al*., 2018).

Ecophysiology studies the physiological mechanism and the interactions between plants and their physical, chemical and biotic environment (Lambers *et al*., 2008). Among these processes, the photosynthetic activity allows to know the behavior and performance of a species and its interaction with environmental conditions (Miguel *et al*., 2007) and to identify the behavior of the plant and its response to specific growth conditions (Rodrigo, 2007).

Niether *et al*. (2018) indicated that the reduction of radiation generated by forest trees improves environmental conditions for cocoa cultivation because cocoa has a low light saturation point. The 95 % maximum photosynthesis occurs with photosynthetically active radiation of 200 μmol $m^{-2} s^{-1}$ (Baligar *et al*., 2008). Cocoa photosynthesis is also sensitive to high vapor pressure deficit (VPD) (Köhler *et al*., 2014), CO_2 assimilated decreases when leaf VPD is higher than 2 kPa (Balasimha *et al*., 1991). Under agroforestry system values for photosynthesis rates (3 to 7 μmol CO_2 $m^{-2} s^{-1}$) and stomatal conductance (0.05 and 0.12 mol CO_2 $m^{-2} s^{-1}$) for cocoa trees are reported (Araque *et al*., 2012; Baligar *et al*., 2008; Jaimez *et al*., 2018; Mielke *et al*., 2005), while in transpiration rate (E), the values vary between 4 and 6 mmol H_2O $m^{-2} s^{-1}$ (Jaimez *et al*., 2018). This study aimed to evaluate the photosynthetic performance of five cocoa genotypes planted in an agroforestry system in the Colombian humid tropical forest.

MATERIAL AND METHODS

Experimental site

The study was conducted at the Research Center El Nus (Agrosavia), San Roque, Antioquia, Colombia (06° 26' 17.2" N, 74° 49' 32.1" W). The weather conditions in 2018 were an average minimum temperature of 18 ºC, an average maximum temperature of 36 °C, average relative humidity of 79 % and annual accumulated rainfall in the region of 2229 mm (Figure 1).

Plant material and experimental design

Five cocoa clones (TCS01, TCS06, TCS13, TCS19 and CCN51) were evaluated under a complete randomized blocks experimental design with four replications; the experimental unit was a group of 20 plants per clone.

Plantation management

Cocoa trees were planted in December 2016 (3 years old) at a distance of 3 × 3 m following a triangle arrangement. The study was carried out in an agroforestry system with permanent trees of abarco (*Cariniana pyriformis* Miers). Agudelo-Castañeda *et al*. (2018) recognized abarco for its potential to regulate incident radiation with less variability; it was considered the forest species to generate a permanent shade on the canopy of cocoa trees. The abarco trees were planted in 2015 (4 years old). At the time of evaluation, they had an average height of 5.1 m and a diameter of 6.04 cm at 1.20 m from the soil surface. The trees were established in 16-m double rows at a distance of 4 × 4 m from each other in a triangle arrangement.

Variables measured

Leaf gas exchange measurements were carried out in September 2018 with a portable photosynthesis measuring system that incorporates an infrared gas analyzer (LCi-ADC Bioscience, Hertfordshire, UK). Measurements were performed on a young, fully expanded leaf of six plants from each cocoa clone for five consecutive days. Net photosynthesis (A, μmol CO_2 $m^{-2} s^{-1}$), transpiration (E, mmol H_2O $m^2 s^{-1}$), stomatal conductance (g_s, mol CO_2 $m^{-2} s^{-1}$) and photosynthetically active radiation (PAR, μmol photons $m^{-2} s^{-1}$) were registered every hour from 08:00 to 17:00 hours. In addition, the A/E and A/PAR ratio, respectively, calculated the instantaneous water use efficiency (WUEi, mmol CO_2 $mmol^{-1}$ H_2O) and radiation use efficiency.

Vapor pressure deficit (VPD, measured in kPa) was calculated from daily air temperature and relative humidity records, according to Equation [1] proposed by Rosenberg *et al*. (1983). The temperature and humidity values were registered with a thermo-hygrometer (Thermo Hygro & Clock, VAPRECISION, Fountain Valley, California, USA) at 30-minute intervals during the day (8:00 to 17:00 hours).

$$VDP = 0.61078 \exp [(17.269 \times T)/(T + 237.3)] \times [(1+(HR/100)] \qquad \text{Ec (1)}$$

An SS1 SunScan Canopy Analysis System (Delta-T Devices Ltd, Cambridge, UK) was used to measure the percentage of incident light intercepted by shade trees

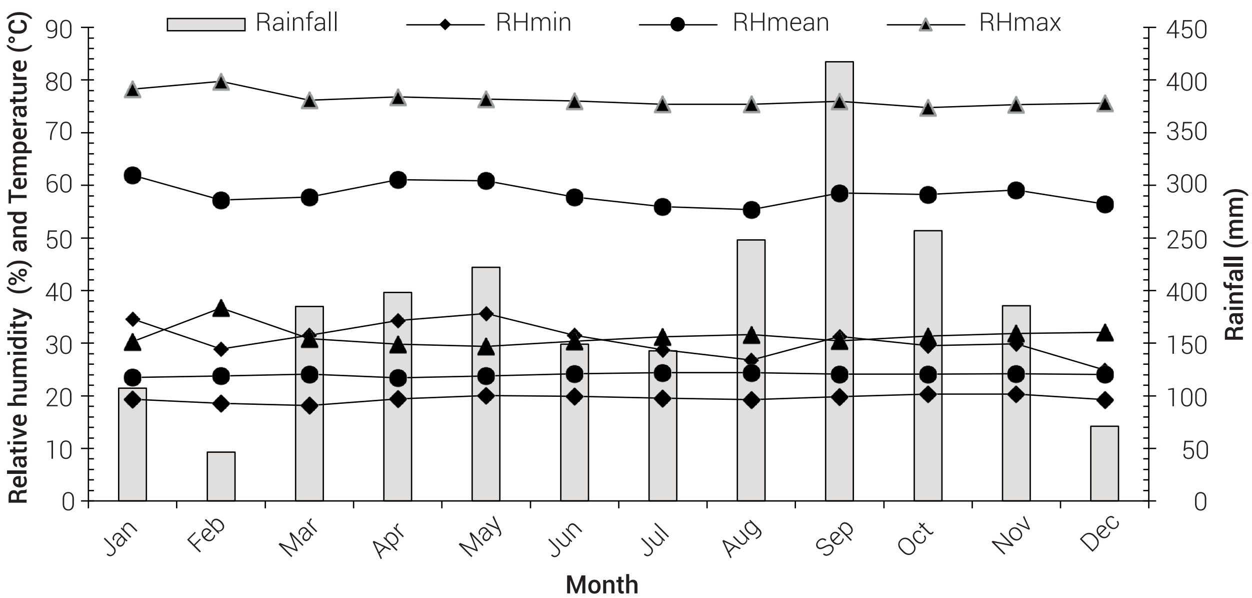

Figure 1. Rainfall (mm), minimum (RHmin), mean (RHmean) and maximum (RHmax) relative humidity (%), minimum (Tmin), mean (Tmean) and maximum (Tmax) temperature. Research Center El Nus (Agrosavia), San Roque, Antioquia, Colombia, 2018.

canopy. Incident radiation flux above the canopy, radiation flux transmitted below the shades tree canopy, extinction coefficient (k) and leaf area index (LAI) were also recorded every half hour, from 08:00 to 17:00.

Daily integral photosynthesis

To determinate the accumulated value throughout the day of the physiological variables A, g_s, E and PAR, the area under the curve (AUC) was estimated for each gas exchange variable by fractioning the total into trapezoidal areas, and the individual areas were calculated using the trapezoid (Equation 2) through a macro of the statistical environment SAS® 9.4 using the analysis referenced by Córdoba *et al*. (2018), which was adapted from routines described by Huang and Xiao (2010) and Shiang (2004).

$$Area = 0.5 \times (m_i + m_{i-1}) \times (t_i - t_{i-1}) \quad \text{Ec.(2)}$$

Where m_i corresponds to the i_{th} measurement, while t_i is the i_{th} time; therefore, the AUC is the sum of all the individual areas estimated (Equation 3).

$$AUC = \sum_{i=2}^{n} 0.5 \times (m_i + m_{i-1}) \times (t_i - t_{i-1}) \quad \text{Ec (3)}$$

Statistical analysis

Analysis of variance of the AUC was performed. The Tukey test separated treatments with statistical differences at P ≤ 0.05 probability. Both analyses were carried out using "*agricolae*" package (Mendiburu, 2020) of the statistical environment (R Core Team, 2020).

RESULTS AND DISCUSSION

Light habitat and vapor pressure deficit

Abarco (*Cariniana pyriformis* Miers) generated a shade level between 9.93 and 20.3 % over cocoa canopy (Figure 2F). Under that condition, the environment temperature below the tree canopy and over cocoa canopy was between 23 and 31 °C and relative humidity was between 42 and 73 %; PAR was between 200 and 1000 μmol photons m^{-2} s^{-1} (Figure 2D). Increasing rates of net photosynthesis (A) have been reported as radiation (PAR) reaches photosynthetic photon flux densities (PPFD) between 300 and 750 μmol photons m^{-2} s^{-1} (Avila *et al*., 2016; Da Matta *et al*., 2001; Mielke *et al*., 2005). Likewise, as shown in Figure 3, the highest incident radiation was concentrated between 200 and 500 μmol photons m^{-2} s^{-1}, near light saturation point for cocoa, which is close to 200 y 500 μmol photons m^{-2} s^{-1}, where plants reach 95 % of the maximum photosynthetic activity. Values close to 1000 μmol photons m^{-2} s^{-1} tend to reduce net photosynthesis. As explained by Baligar

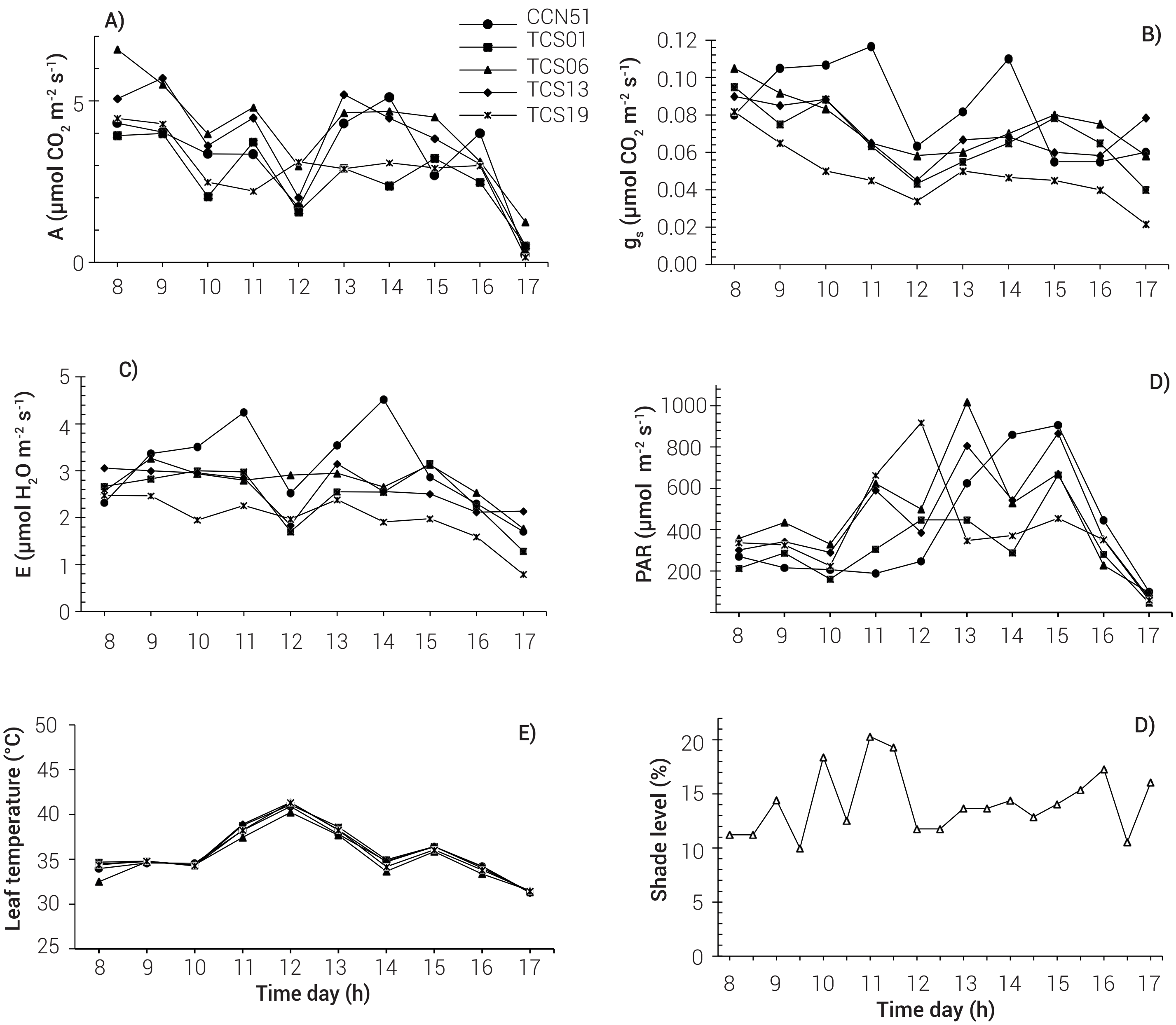

Figure 2. Diurnal variation of gas exchange parameters of five cocoa clones. A) net photosynthesis, B) stomatal conductance, C) transpiration, D) photosynthetically active radiation, E) leaf temperature, F) shade level at the top of *Theobroma cacao* trees.

et al. (2008), the photosynthetic speed decreases if the photosynthetic apparatus is exposed to PPFD higher than 1800 µmol m^{-2} s^{-1}; thus, the exposure affects the photosynthetic apparatus of the cocoa leaf (Raja Harun and Hardwick, 1988; cited by Almeida and Valle 2007).

According to Agudelo-Castañeda *et al*. (2018), abarco, as a forest species of the system, has been recognized for its potential to regulate incident radiation with less variability. It allows cocoa plants to achieve higher rates of photosynthesis (5.39 µmoles CO_2 m^{-2} s^{-1}) because this species has the lowest percentage of natural leaf removal in the dry season. The aforementioned allows maintaining radiation levels suitable for cocoa cultivation throughout the year, compared to other species such as teak (*Tectona grandis* L. f.- Verbenaceae) and rubber [*Hevea brasiliensis*

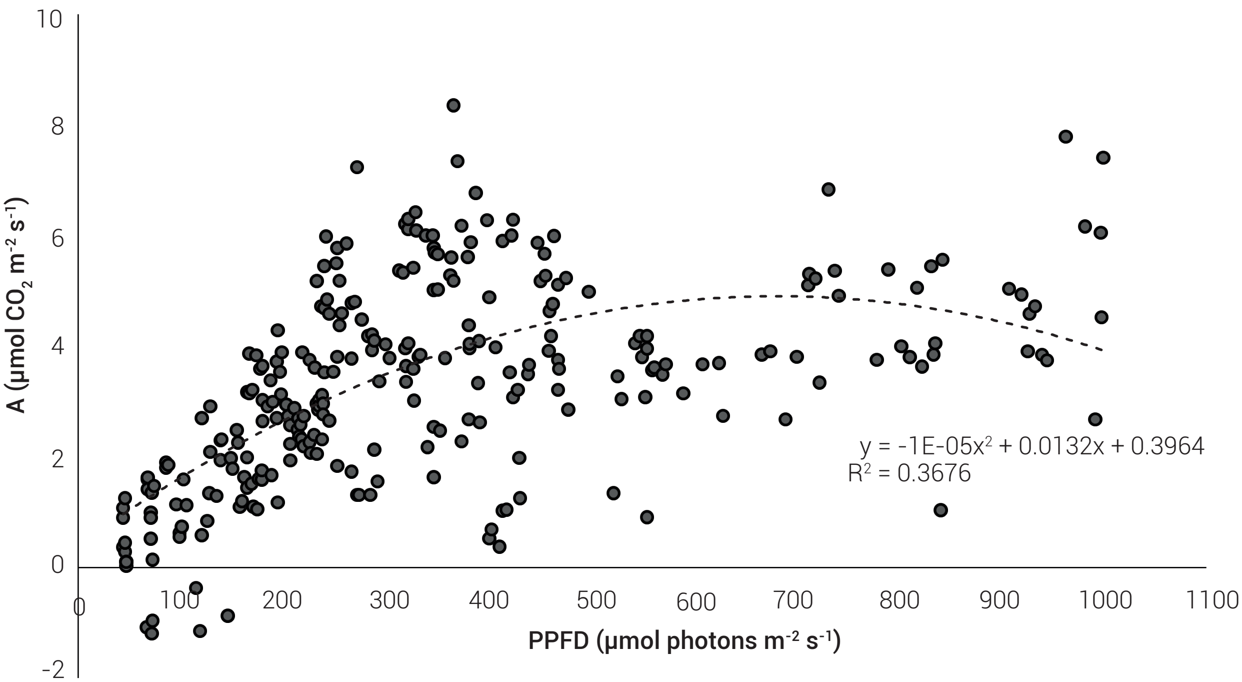

Figure 3. Net photosynthetic rate (A) and photosynthetic photon flux density (PPFD) relation of five *Theobroma cacao* clones planted in an agroforestry system.

(Willd. ex A. Juss.) Müll Arg. - Euphorbiaceae].

The VDP reached the lowest values in the arly hours of the day (0.25 to 0.45 kPa) and in the late afternoon (0.43 to 0.3 kPa), while the highest VDP values (0.71 kPa) were registered when the temperature reached 30 °C and a relative humidity of 40 % (Figure 4). Mielke *et al*. (2005) indicated that high VPD values could induce stomatal closure and limit the entry and diffusion of CO_2; thus, the highest VPD near noon can explain the stomatal closure between 11:00 and 14:00 hours and, consequently, the decrease in photosynthetic activity. Similar results were found by Almeida *et al*. (2014), who stated that the reduction in CO_2 assimilation is associated with the stomatal closure by increasing VPD.

Gas exchange

All cocoa clones, except for CCN51, reached their highest photosynthetic capacity between 08:00 and 09:00 hours, showing a maximum A of 6.6 µmol CO_2 m^{-2} s^{-1} (TCS06), 5.72 µmol CO_2 m^{-2} s^{-1} (TCS13), 4.46 µmol CO_2 m^{-2} s^{-1} (TCS19) and 3.99 µmol CO_2 m^{-2} s^{-1} (TCS01), while at noon, a reduction in photosynthetic activity was observed to rates of 2.98 µmol CO_2 m^{-2} s^{-1} (TCS06), 2.01 µmol CO_2 m^{-2} s^{-1} (TCS13), 3.12 µmol CO_2 m^{-2} s^{-1} (TCS19) and 1.57 µmol CO_2 m^{-2} s^{-1} (TCS01); after this decreasing, clone TSC19 was the only one that remained constant in the rate of CO_2 fixation with values close to 3.12 µmol CO_2 m^{-2} s^{-1} in the afternoon, unlike clones TCS13 and CCN51, which for the afternoon showed an increase of A, reaching values of 5.20 and 5.12 µmol CO_2 m^{-2} s^{-1}, respectively (Figure 2A).

In general, the photosynthetic rates observed in this study were between 3.0 and 8.8 µmol CO_2 m^{-2} s^{-1}, similar to those reported by several authors (Almeida *et al*., 2014; Araque *et al*., 2012; Jaimez *et al*., 2018; Mielke *et al*., 2005; Ribeiro *et al*., 2016;). Cocoa has a relatively low net assimilation rate (A) compared to other crops (Alvim, 1977). Cocoa A coincides with those reported for C_3 plants, as the case of coffee (*Coffe arabica*), with low values of A (3 to 12 µmol CO_2 m^{-2} s^{-1}) and lower than C_4 plants, such as maize (*Zea mays* L.), with A values of 60 to 90 µmol CO_2 m^{-2} s^{-1} (Polanía *et al*., 1982).

According to Agudelo *et al*. (2018), clones TSC19 and TCS13 showed the highest photosynthetic rate (4.8 and 4.36 µmoles CO_2 m^{-2} s^{-1}, respectively) between 08:00 and 12:00 hours, compared to other regional clones, such as ICS95. In this study, clones TCS13 and TCS06 stood out by their photosynthetic performance. Regarding g_s and E, a reduction towards noon was observed, similar to the A trend (Figure 2B and 2C). The g_s values ranged from 0.03 (TCS19) to 0.06 mol CO_2 m^{-2} s^{-1} (TCS06 and CCN51). In

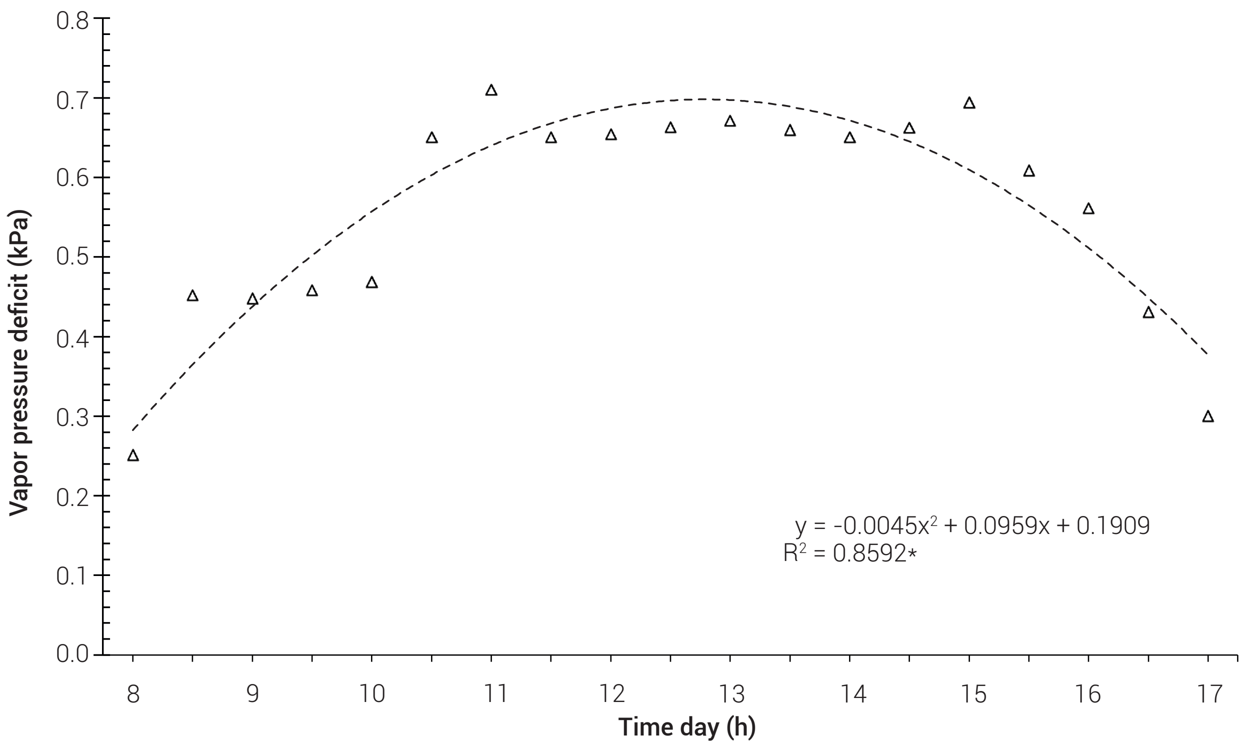

Figure 4. Vapor pressure deficit (VPD) at the top of *Theobroma cacao* plants. *: P ≤ 0.05.

terms of E, values of 1.70 (TCS01) to 2.91 mmol H_2O m^{-2} s^{-1} (TCS06) were recorded, coinciding with the time when the highest leaf temperature occurred (41 °C) (Figure 2E).

Throughout the day, clone CCN51 stood out for presenting the highest values of g_s at 11:00 and 14:00 hours (0.12 and 0.11 mol CO_2 m^{-2} s^{-1}, respectively) and, consequently, higher E (4.25 and 4.52 mmol H_2O m^{-2} s^{-1}, respectively); however, clone CCSN51 did not show the highest photosynthetic activity. Several g_s are reported, ranging from 0.02 to 0.14 mol CO_2 m^{-2} s^{-1} (Araque *et al*., 2012; Baligar *et al*., 2008; De Almeida *et al*., 2016; Jaimez *et al*., 2018; Mielke *et al*., 2005), while transpiration ranged between 0.39 and 6.0 mmol H_2O m^{-2} s^{-1}. Mielke *et al*. (2005), Baligar *et al*. (2008), Almeida *et al*. (2014) and Jaimez *et al*. (2018) found a direct relationship between the values of g_s and A, the greater the conductance, the greater the photosynthesis (g_s 0.03 mol CO_2 m^{-2} s^{-1} / A 4 µmoles CO_2 m^{-2} s^{-1} and g_s 0.13 mol CO_2 m^{-2} s^{-1} / A 8.5 µmoles CO_2 m^{-2} s^{-1}). On the other hand, Araque *et al*. (2012) found that variation in the parameters A, g_s and E was related to the moisture regime; all cultivars exhibited a 60 % reduction in g_s with drought, with a 73 % decrease in A and E; meanwhile, De Almeida *et al*. (2016) reported a positive relationship between the values of g_s and A, but in turn, these were influenced by different water levels.

Jaimez *et al*. (2018) showed that g_s, unlike E, is influenced by PAR variation, and that high values of g_s resulted in a greater CO_2 availability for carboxylation by Rubisco during the Calvin cycle; in contrast, Baligar *et al*. (2008) indicated that the main factor influencing E is VPD, presenting a direct relationship between both parameters.

The daily integral of gas exchange (Area under the curve-AUC)

There were variaritions for cocoa clones (P ≤ 0.01) in the daily integral of A, g_s and E. The highest values of these parameters were found in clones TCS06, TCS13 and CCN51, while the lowest ones were found in clone TCS19. Clone TCS06 presented 137,300 µmol CO_2 m^{-2} d^{-1}, corresponding to a variation of 44 and 40 % more CO_2 fixed than clones TCS19 and TCS01, respectively. A similar behavior was observed for E; clone CCN51 showed significantly (P = 0.00299) more stomatal conductance and transpiration rates than clone TCS19, reaching values of 108,300 mmol H_2O m^{-2} d^{-1} compared to 66,730 mmol H_2O m^{-2} day^{-1} in TCS19. Regarding g_s, clone TCS19 showed the lowest value (1484 mol CO_2 m^{-2} d^{-1}), which was significantly different for the stomatal conductance reached by the remaining four clones. Regarding the efficiency of the use of water and radiation, the clones did not differ significantly (P > 0.05) (Table 1). Agudelo *et al*. (2018) reported that clone TCS13 achieved a greater efficient use of water (2.8 µmol

Table 1. The daily integral of net photosynthetic rate (A), transpiration rate (E), stomatal conductance (g_s), water use efficiency (WUE), and radiation use efficiency (RUE) measured in leaves of five Theobroma cacao clones planted in an agroforestry system.

Parameter	Clones										p Value
	TCS19		TCS06		CCN51		TCS13		TCS01		
A (µmol CO_2 m^{-2} d^{-1})	94,820	b	137,300	a	111,100	ab	126,600	ab	97,990	b	0.00507**
E (mmol H_2O m^{-2} d^{-1})	66,730	b	92,920	ab	108,300	a	81,330	ab	91200	ab	0.00299**
g_s (mol CO_2 m^{-2} d^{-1})	1,488	b	2,394	a	2,748	a	2,235	a	2398	a	0.00186**
PAR (µmol photons m^{-2} d^{-1})	13,880	b	20,530	a	13,960	b	1,5710	b	12860	b	0.0177*
WUE (A/E)	1.418	a	1.472	a	1.072	a	1.615	a	1.133	a	0.0354*
RUE (A/PAR)	0.0075	a	0.0067	a	0.0087	a	0.0082	a	0.0080	a	0.395ns

Means followed by the same letter in the rows are not significantly different (Tukey, P ≤ 0.05) *: P ≤ 0.05, **: P ≤ 0.01, ns: no statistical significance.

CO_2 $mmol^{-1}$ H_2O) compared to clones like ICS95, which coincides with findings in this study, where the clone TCS13 reached the highest values in WUE (1.6 µmol CO_2 $mmol^{-1}$ H_2O).

Regarding the selection of forest species used as shade for cocoa trees, it is necessary to consider the climatic, edaphic and environmental requirements, and some morphophysiological characteristics that affect the percentage and durability of the shade on the cocoa canopy; among them, the foliar density and the longevity of the leaves (Chavarría, 2013).

Agudelo-Castañeda *et al*. (2018) stated that trees used in agroforestry systems influence the ecophysiological behavior of cocoa clones, mainly in photosynthesis rates. Abarco has greater benefits, especially in wet and dry seasons, which allows maintaining the physiological activity of cocoa throughout the year; thus abarco is a recommended tree for the studied region, which has two rainy seasons (March-June and September-November) and two periods of less precipitation (December-February and July-August). In this sense, in cocoa-producing areas with high radiation intensity and water-deficient seasons, abarco can be helpful in agroforestry arrangements with cocoa; however, in the cocoa-producing areas with high cloudiness and periods without water deficit, abarco can be a forest alternative. Greater spacing between forest components should be considered to guarantee adequate shade and radiation availability to carry out photosynthesis. Similarly, the cocoa-abarco spacing arrangement needs to consider the light requirements of the cocoa genetic material to be used. As observed in this study, clone CCN51 requires more radiation to achieve high photosynthetic rates, unlike clones TCS 01, TCS 06 and TCS 19, which achieved higher values of fixed carbon at lower radiation.

CONCLUSIONS

Clone TCS06 presented the highest photosynthesis as a function of the daily integral of gas exchange compared to TCS19 and TCS13. Vapor pressure deficit (VPD) regulates gas exchange behavior in terms of g_s and E. The most stable material in stomatal conductance, with variations in VPD, was TCS06 compared to clone TCS19. Clone CCN51 requires higher photosynthetically active radiation (PAR) to achieve maximum photosynthetic activity, unlike that observed in other TCS clones, where its performance is obtained with lower PAR values.

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