DOI: 10.1007/s11099-013-0048-8 PHOTOSYNTHETICA 52 (1): 16-21, 2014

Leaf gas exchange in species of the *Theobroma* genus

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Abstract

Species of the *Theobroma* genus are primarily known by their commercially valuable seeds, especially, *T. cacao* is one of the most important tropical perennial crops. Beside *T. grandiflorum*, *T. bicolor*, and *T. angustifolium*, *T. cacao* is the only species of the genus that has been better studied to obtain physiologically relevant information. The main objective of this work was to evaluate the leaf gas exchange in seedlings of seven species of the *Theobroma* genus, seeking to identify characteristics that could be used in *T. cacao* breeding programmes. The study was realized under greenhouse conditions using six-month-old seedlings, in which net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration (E), as well as parameters derived from light curves (P_N *vs.* photosynthetically active radiation) were evaluated. *T. cacao,* along with *T. microcarpum,* showed the lowest values of P_N, g_s, and E, while the highest values were presented by *T. speciosum*, which showed higher saturation irradiance and lower intrinsic and instantaneous water-use efficiencies, being considered the species less conservative in water use. Therefore, the parameters shown by the different evaluated species could serve to design *T. cacao* genotypes, through introgression of genes for specific environments such as the cabruca system widespread in southern Bahia, Brazil.

Additional key words: light-response curves; photosynthesis; stomatal conductance; transpiration; water-use efficiency.

Introduction

The success of any breeding or conservation programme of plant species depends on the knowledge of inter-specific variations of interest, including the wild relatives. Most agronomic traits are influenced by environmental factors, showing continuous variation and high degree of phenotypic plasticity. A major challenge for plant breeding programmes is the inclusion of important agronomic traits of wild germplasm in selected materials. In the case of cacao, wild species have not been used in breeding and are not even present in many germplasm collections (Santos 2011).

Several strategies have been used to access the genetic variability in germplasm collections of *T. cacao* as morphological and molecular markers (Lerceteau *et al.* 1997, Lachenaud *et al.* 1999, Charters and Wilkinson 2000, Faleiro *et al.* 2002, Santos *et al.* 2011). All Amazonian species of the *Theobroma* genus produce fruits of commercial value (Ducke 1953, Calzavara *et al.* 1984, Venturieri and Aguiar 1988). However, *T. grandiflorum*, *T. bicolor*, *T. angustifolium,* and *T. cacao* are the only cultivated species, while *T. cacao* is the only one that reaches also high expression as a crop (Baker *et al.* 1954, Reksodihardjo 1964). Natural hybrids between *Theobroma* species are extremely rare. There is only one report describing the discovery of one tree with characteristics intermediate between *T. obovatum* and *T. subincanum*, suggesting its possible hybrid origin (Reksodihardjo 1964) at Caquetá River (Baker *et al.* 1954) in Remolino forest (southwestern Colombia). Furthermore, Reksodihardjo (1964) mentioned the existence of other exsiccates of hybrids between *T. obovatum* and *T. subincanum* deposited in the herbaria of ICTA, Trinidad, and of the Institute of Natural Sciences in Bogota, Colombia. He also mentioned two exsiccates of this cross in Brazil, deposited in the herbarium of the extinct IAN (current EMBRAPA

Received 13 June 2012, *accepted* 8 March 2013.

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Abbreviations: E – transpiration; g_s – stomatal conductance to water vapor; g_s/VPD – stomatal conductance to water vapor normalized for leaf-to-air vapor pressure deficit; I_c – compensation irradiance; I_s – saturation irradiance; PAR – photosynthetically active radiation; P_{Gmax} – maximum rate of gross photosynthetic rate at saturation irradiance; P_N – net photosynthetic rate per leaf area unit; R_D – dark respiration rate; VPD – leaf-to-air vapor pressure deficit; WUE ($=P_N/E$) – instantaneous water-use efficiency; WUE_i ($= P_N/g_s$) – intrinsic water-use efficiency.

Amazônia Oriental). There are also two other exsiccates of a probable natural hybrid between *T. glaucum* and *T. sylvestre* deposited in the EMBRAPA Amazonia Oriental herbarium (Addison and Tavares 1951).

A few decades ago, it was thought that hybridization between species of different sections would be extremely difficult, if not impossible. However, hybrids were obtained between the sections Glossopetalum and Andropetalum (*T. angustifolium* × *T. mammosum* and *T. simiarum* × *T. mammosum*) (Cuatrecasas 1964, Reksodihardjo 1964). In 1966, a hybrid between *T. grandiflorum* (section Glossopetalum) and *T. cacao* (Theobroma section) was also obtained (Martinson 1966). More recently, Faleiro *et al.* (2003) obtained a new hybrid between *T. cacao* × *T. grandiflorum,* which was confirmed by RAPD markers. These hybrids indicate a possibility of introgression into *T. cacao* of divergent genes for agronomic traits of interest such as precocity, quality traits, and disease resistance from wild species.

Therefore, several photosynthetic parameters, which could be used to design plants for specific environments, were shown in this work. It is well known in Bahia, Brazil that most cacao plantations are grown under an agrosystem locally called cabruca. In this system, cacao is planted in the understory compartment, shaded by trees of the Atlantic Forest. One of the problems in this system is low irradiation and the inefficiency of the current genotypes to use it properly. Thus, the gene introgression from some species evaluated in this work could be done to design a more suitable cacao plant for such an environment.

The objective of our study was to evaluate leaf gas-exchange parameters in seedlings of different *Theobroma* species, seeking to identify characteristics, which might be useful in *T. cacao* breeding programmes.

Materials and methods

Plant material and growth conditions: Seven *Theobroma* species were evaluated; two, which are cropped in a commercial scale (*i.e. T. cacao* and *T. grandiflorum*), and five wild species (*T. subincanum, T. bicolor, T. obovatum, T. microcarpum*, and *T. speciosum*). The species are deposited in one of CEPLAC germplasm banks, at the Experimental Station José Haroldo, Marituba, Pará, Brazil, and in the germplasm bank of the Agronomic Research Center of the Humid Tropics, Belém, Pará, Brazil. The experiment was conducted in a greenhouse at the Universidade Estadual de Santa Cruz – UESC, Ilhéus, Bahia, Brazil (14º47'S, 39º16'W, 55 m a.s.l.) under irradiance of 760 ± 84 μmol m^{-2} s^{-1} PAR, temperature of 26 ± 1°C, and relative humidity of 84 ± 3%, from March to August 2009. Seeds were obtained from open pollination pods and planted in polyethylene pots filled with 12 kg of soil (Alfisol, series Cepec), enriched with mineral macro- and micronutrients according to the nutritional needs of *T. cacao* (Souza 2007). The planted pots were kept under a 50%-sunlight shade sheath and watered to a field capacity twice a week.

Photosynthetic parameters were evaluated 6 months after germination. Leaf gas-exchange measurements were done on mature leaves of 5 plants of each *Theobroma* species, always between 07:30 and 12:00 h, using a portable photosynthesis system *Li-6400* (*Li-Cor*, Nebraska, USA) equipped with an artificial light source *6400-02B RedBlue (Li-Cor*, Nebraska, USA). Photosynthetically active radiation (PAR) response curves were done at ten levels (800, 600, 400, 200, 100, 50, 25, 10, 5, and 0 μmol m^{-2} s^{-1} PAR). The sequence was always initiated in decreasing order of PAR levels. The minimum pre-established time for reading stabilization was set at 60 s at each PAR level and the maximum to save each reading, at 120 s, as well as a maximum admitted coefficient of variation of 0.3%. Block temperature was maintained constant at 26°C using the equipment coolers, the CO_2 concentration and air relative humidity inside the chamber varied from 370 to 390 μmol mol^{-1} and 59 to 60%, respectively. Instantaneous leaf gas-exchange measurements followed the same patterns as the measurements for the P_N *vs.* PAR curves. P_N, g_s, and E were estimated from differences in CO_2 and air humidity values inside the chamber, as determined by the infrared gas analyzer. Intrinsic water-use efficiency (WUE_i), instantaneous water-use efficiency (WUE), and stomatal conductance to water vapor normalized for leaf-to-air vapor pressure deficit (g_s/VPD) were also calculated.

Statistics: Parameters derived from light-saturation curves were estimated for each individual using nonlinear regression for the exponential equation: $P_N = P_{Gmax} [1 - \exp(-\alpha \text{ PAR}/P_{Gmax})] - R_D$, where P_{Gmax} is the maximum rate of gross photosynthetic rate at saturation irradiance, α is the apparent quantum efficiency of photosynthesis, and R_D is the dark respiration rate (Iqbal *et al.* 1997). For each individual replicate, saturation irradiance (I_s) was estimated from the exponential equations, when $P_N = 0.9\ P_{Gmax}$ and the compensation irradiance (I_c, x-intercept), estimated by a linear regression of the first points of the P_N *vs.* PAR (Escalona *et al.* 1999). The photosynthetic parameters were compared using *Fisher*′s Least Significant Difference test at 5% probability, when significance was shown by the *F* test in the analysis of variance.

Results

There were interspecific variations in P_N, g_s, and E. The highest values of these parameters were found in *T. speciosum*, while the lowest ones in *T. cacao* and *T. microcarpum*, ranging between 3.5–8.8 μmol(CO_2) m^{-2} s^{-1} for P_N, 0.023–0.108 mol(H_2O) m^{-2} s^{-1} for g_s, and 0.39–1.63 mmol(H_2O) m^{-2} s^{-1} for E, corresponding to interspecific variations of 151, 370, and 318%, respectively (Table 1).

T. cacao and *T. microcarpum* showed higher values of WUE$_i$ and WUE and lower ones of g_s/VPD (Table 2). The lower values of WUE$_i$ and WUE were shown by *T. subinancum*, *T. obovatum*, and *T. speciosum*. There was a 91% variation in WUE$_i$ values ranging from 86.4 in *T. speciosum* to 165.4 μmol(CO_2) mol(H_2O)$^{-1}$ in *T. cacao* and *T. bicolor*, respectively. However, the interspecific variation of WUE was only 69% (6.1 to 10.3 [μmol(CO_2) mmol(H_2O)$^{-1}$]). The g_s/VPD values ranged between 0.014–0.070 mol kPa^{-1} in *T. cacao* and *T. speciosum*, respectively (Table 2). In graphs (data not shown) adjusted for P_N *vs.* g_s and E *vs.* g_s, direct relationships were observed for all species. For the relations VPD *vs.* g_s and VPD *vs.* E, however, the influence was not equal (data not shown), while g_s and E remained constant in *T. microcarpum*, and g_s increased almost linearly in *T. speciosum*.

Table 1. Net photosynthetic rate (P_N), stomatal conductance to water vapor (g_s), and transpiration rate (E) measured at saturating irradiance (PAR > 400 μmol m^{-2} s^{-1}) in leaves of seven *Theobroma* species. Each value represents mean (± SE) of five replicates. Means followed by *the same letter* did not differ by the LSD test ($p<0.05$).

Species	P_N [μmol(CO_2) m^{-2} s^{-1}]	g_s [mol(H_2O) m^{-2} s^{-1}]	E [mmol(H_2O) m^{-2} s^{-1}]
T. subincanum	5.8 ± 0.5^{b}	0.060 ± 0.008^{b}	0.91 ± 0.09^{b}
T. cacao	3.7 ± 0.2^{cd}	0.023 ± 0.003^{c}	0.39 ± 0.06^{c}
T. bicolor	5.2 ± 0.3^{b}	0.040 ± 0.007^{bc}	0.67 ± 0.11^{bc}
T. grandiflorum	5.4 ± 0.1^{b}	0.043 ± 0.002^{bc}	0.70 ± 0.03^{bc}
T. obovatum	4.8 ± 0.5^{bc}	0.050 ± 0.015^{bc}	0.83 ± 0.32^{bc}
T. microcarpum	3.5 ± 0.1^{d}	0.025 ± 0.002^{c}	0.46 ± 0.05^{c}
T. speciosum	8.8 ± 0.5^{a}	0.108 ± 0.013^{a}	1.63 ± 0.26^{a}

Table 2. Intrinsic (WUE$_i$ = P_N/g_s) and instantaneous (WUE = P_N/E) water-use efficiency and stomatal conductance to water vapor normalized for leaf-to-air vapor pressure deficit (g_s/VPD), calculated from values measured at saturating irradiance (PAR > 400 μmol m^{-2} s^{-1}) in leaves of seven *Theobroma* species. Each value represents mean (± SE) of five replicates. Means followed by *the same letter* did not differ by the LSD test ($p<0.05$).

Species	P_N/g_s [μmol(CO_2) mol^{-1}(H_2O)]	P_N/E [μmol(CO_2) mmol^{-1}(H_2O)]	g_s/DPV [mol(H_2O) kPa^{-1}]
T. subincanum	101.1 ± 6.0^{c}	6.5 ± 0.5^{b}	0.040 ± 0.007^{b}
T. cacao	165.4 ± 14.2^{a}	10.3 ± 1.3^{a}	0.014 ± 0.001^{d}
T. bicolor	143.3 ± 15.3^{ab}	8.4 ± 0.8^{ab}	0.024 ± 0.004^{cd}
T. grandiflorum	127.2 ± 5.3^{b}	7.8 ± 0.5^{ab}	0.026 ± 0.001^{bc}
T. obovatum	107.4 ± 17.3^{bc}	7.0 ± 1.5^{b}	0.030 ± 0.006^{bc}
T. microcarpum	146.0 ± 12.1^{ab}	7.9 ± 1.0^{ab}	0.014 ± 0.002^{d}
T. speciosum	86.4 ± 8.8^{c}	6.1 ± 0.9^{b}	0.070 ± 0.005^{a}

Table 3. Light-response curve parameters estimated after fitting an exponential model to the measured data of P_N *vs.* PAR in leaves of seven *Theobroma* species. P_{Gmax} – light-saturated gross photosynthetic rate; α – apparent quantum efficiency of photosynthesis; R_D – dark respiration rate; I_c and I_s – compensation and saturation irradiances, respectively. Each value represents mean (± SE) of five replicates. Means followed by *the same letter* did not differ by the LSD test ($p<0.05$).

Species	P_{Gmax} [μmol(CO_2) m^{-2} s^{-1}]	α [μmol(CO_2) μmol(photon)$^{-1}$]	R_D [μmol(CO_2) m^{-2} s^{-1}]	I_c [μmol(photon) m^{-2} s^{-1}]	I_s [μmol(photon) m^{-2} s^{-1}]
T. subincanum	6.2 ± 0.7^{b}	0.053 ± 0.004^{ab}	0.22 ± 0.06^{a}	3.95 ± 1.01^{a}	285± 46^{abc}
T. cacao	4.0 ± 0.2^{d}	0.052 ± 0.016^{ab}	0.37 ± 0.17^{a}	5.85 ± 2.26^{a}	235 ± 59^{bc}
T. bicolor	5.3 ± 0.5^{bcd}	0.036 ± 0.006^{b}	0.09 ± 0.04^{a}	2.26 ± 0.72^{a}	369 ± 81^{ab}
T. grandiflorum	5.9 ± 0.2^{c}	0.068 ± 0.007^{a}	0.41 ± 0.17^{a}	5.73 ± 1.97^{a}	237 ± 35^{bc}
T. obovatum	5.1 ± 0.4^{bcd}	0.061 ± 0.013^{ab}	0.32 ± 0.27^{a}	4.11 ± 2.97^{a}	211 ± 33^{c}
T. microcarpum	3.6 ± 0.2^{d}	0.048 ± 0.005^{ab}	0.17 ± 0.06^{a}	3.76 ± 1.57^{a}	179 ± 16^{c}
T. speciosum	9.4 ± 0.8^{a}	0.054 ± 0.004^{ab}	0.30 ± 0.07^{a}	5.38 ± 0.88^{a}	407 ± 32^{a}

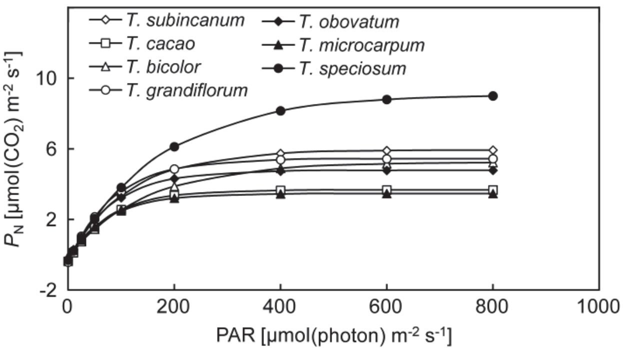

Fig. 1. Light-response curves of photosynthesis in leaves of seven *Theobroma* species. Each curve represents modeled responses after fitting an exponential model to measured data of P_N *vs.* PAR in five replicates. *See* the text for light response and modeling procedures.

Based on the P_N *vs.* PAR response curves, it was found that the higher values of P_{Gmax} and I_s were shown by *T. speciosum* [P_{Gmax} = 9.4 μmol(CO$_2$) m^{-2} s^{-1} and I$_s$ = 406.5 μmol(photon) m^{-2} s^{-1}] and the lowest by *T. microcarpum* [P_{Gmax} = 3.6 μmol(CO$_2$) m^{-2} s^{-1} and I_s = 179.0 μmol(photon) m^{-2} s^{-1}]. However, the lowest and highest values of α were presented by *T. grandiflorum* and *T. bicolor*, respectively. There was no significant interspecific variation ($p<0.05$) for R_D and I_c values (Fig. 1 and Table 3).

Discussion

Interspecific variations in photosynthetic rates of the *Theobroma* genus were expected, since there are intraspecific variations in the most studied *T. cacao* (Almeida and Valle 2007, 2009). The interspecific differences in P_N found in this study [3.5–8.8 μmol(CO$_2$) m^{-2} s^{-1}] were similar to variations found in other genera, such as species of the *Coffea* genus, which had photosynthetic rates ranging between 4–11 μmol(CO$_2$) m^{-2} s^{-1} (Franck and Vaast 2009). Photosynthetic rates for intraspecific *T. cacao* showed variations from 1 to 7 μmol(CO$_2$) m^{-2} s^{-1} (Mielke *et al*. 2005, Baligar *et al*. 2008, Daymond *et al*. 2011).

Changes in g_s are associated with the control of water loss and CO$_2$ assimilation (Klich 2000). Therefore, P_N and E are linearly related to g_s in all species at low values (Lambers *et al.* 2008). This relationship controls the availability of CO$_2$ and the entry and exit of water vapor through the stomata opening (Ribeiro *et al.* 2009). According to Daley *et al*. (1989), the decrease in g_s may restrict the rate of CO$_2$ fixation with the consequent reduction of its concentration in the substomatal cavities and intercellular spaces. The values of g_s and E observed in *T. cacao* were similar to those found in previous studies with the same species (Mielke *et al*. 2005, Baligar *et al.* 2008), being lower compared with other species of the genus. However, Daymond *et al*. (2011) found wider variation of g_s and E in *T. cacao*, similar to the interspecific variation found in the *Theobroma* genus; the reason for this variation may be the larger number of *T. cacao* genotypes analyzed. According to Farquhar (1978), E decreases with the increase in VPD, suggesting that the stomata are sensitive to environmental changes. Typically, high VPD values constitute one of the main limiting factors for photosynthesis (Costa *et al.* 2001) due to P_N reduction and increased photorespiration, probably by VPD effects in stomata closure, which leads to reduction of internal CO$_2$.

The highest WUE$_i$ and WUE values observed in *T. cacao* and *T. microcarpum* demonstrated that these species have greater g_s control and consequently higher water-use efficiency (Karatassiou and Noitsaki 2010) compared to the other species evaluated. Although *T. speciosum* showed larger P_N, g_s and E remained high, making this species hardly conservative in relation to water use. According to Massonnet *et al.* (2007), WUE$_i$ is less dependent on abiotic factors in several species and it is under a strong genetic control. It could be used as a good selection criterion for introgression of genes, while WUE shows higher dependence on environmental conditions such as temperature, irradiance, and relative humidity. The relationship established between g_s and VPD are in agreement with the discussion regarding WUE, as the less conservative species in water use does not respond to VPD by stomata closing. Although VPD had a strong influence on g_s in many species (Bobich *et al.* 2010), this fact was not evident for the species described here. It was observed in *T. microcarpum* and *T. grandiflorum* that g_s remained constant with increasing VPD. Thus, these species should be susceptible to severe dehydration and less tolerant to soil water stress (Gao *et al.* 2002).

T. speciosum showed higher P_{Gmax} and I_s, while *T. microcarpum* and *T. cacao* had the lowest values (Table 3). The variable I_s distinguishes plant behavior in connection with the utilization of irradiance and reflects the maximum values of PAR radiation, to which the leaves of a plant have been exposed during its development. The PAR values, in which saturation (I_s) occurs, are substantially lower in plants growing in shade than those of plants grown in full sun. In general, growth of plants at low PAR involves reductions in P_{Gmax}, I_s, R_D, and I_c (Straus-Debenedetti and Bazzaz 1996). Plants grown under high irradiation maximize their carbon gain by increasing those parameters and photoprotection capacity (Chazdon *et al.* 1996, Bazzaz 1998). Therefore, leaves under high irradiance show the photosynthetic process characterized by high electron transport capacity and CO_2 fixation (Adams *et al.* 1999).

In general, the higher P_{Gmax}, the greater the gain in photosynthesis. Plants grown under high irradiance are usually able to use better the available radiation (Vats *et al.* 2002). Moreover, shade-grown plants have a photosynthetic gain equal to 1/2–1/3 of the gain of sun-grown plants. These characteristics reflect a survival strategy developed by plants adapted to limited irradiance, in which an increase in the efficiency of CO_2 uptake, better use of the available radiation, and low growth rates occur (Gonçalves *et al.* 2005).

The low I_c values were associated with lower R_D values (Table 3). The values of R_D and I_c found for *T. cacao* [0.37 μmol(CO_2) m^{-2} s^{-1} and 5.85 μmol(photon) m^{-2} s^{-1}, respectively] were different from those observed by Daymond *et al.* (2011) in various *T. cacao* genotypes, which ranged between 0.45–1.39 μmol(CO_2) m^{-2} s^{-1} and 11.7–24.7 μmol(photon) m^{-2} s^{-1}, respectively. Under field conditions, Mielke *et al.* (2005) found R_D values of 1.24 and 1.19 μmol(CO_2) m^{-2} s^{-1} in adult plants of *T. grandiflorum* and *T. cacao*, respectively, which were higher than those observed in this work (Table 3).

Physiologically, low I_c values allow plants to obtain a positive carbon balance under extremely low light intensities. Lower values of R_D contribute significantly to the decrease of I_c and represent a basic adaptation for plant survival under shade. Shade leaves optimize light absorption under low irradiance and reach I_c and I_s much faster (Givinish 1988, Pearcy 2000). Similarly, photosynthesis requires maximization of light absorption together with reduced losses through respiration and/or photorespiration (Zhang *et al.* 2003). Plants grown in an environment with greater light availability show higher P_N, g_s, and I_s values and increased capacity of photoprotection (Chazdon *et al.* 1996, Bazzaz 1998); *T. speciosum* and *T. subincanum* showed such traits in comparison with the other *Theobroma* species evaluated.

Lower R_D and I_c values, evident mainly in *T. bicolor* and *T. microcarpum*, are considered as prerequisites for a successful acclimation of plants to low irradiance, because leaves must be able to capture the available light and convert it into chemical energy with the highest possible efficiency (Cao 2000, Feng *et al.* 2004). The variation of R_D is a necessary maintenance process for survival; therefore, being light a limiting factor for carbon gain and growth under shade, the cost of plant growth is generally lower (Sims and Pearcy 1991). Thus, the photosynthetic apparatus of crop species reflects the selection pressure for maximal light absorption under low irradiance, while minimizing the respiratory cost associated with high photosynthetic capacity (Chazdon *et al.* 1996). On the other hand, these features can also interact adversely further increasing the susceptibility of plants to stress caused by irradiance (Schiefthaler *et al.* 1999, Guo *et al.* 2006).

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