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Morphoanatomic and Physiological Characterization of Cacao (*Theobroma cacao* L.) Genotypes in the South of Bahia, Brazil

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Abstract: Cocoa tree genotypes (*Theobroma cacao* L.) were studied and characterized in terms of their morphoanatomical and physiological attributes in a non-stressful environment, as these attributes are of fundamental importance to understanding the plant's relationship with the environment. Therefore, the objective of this study is to describe morphoanatomical and physiological patterns that can differentiate the seven cocoa genotypes, evaluated under the same conditions of temperature, humidity, and light. The genotypes remained in a greenhouse for 40 days, where sample collection procedures were carried out to analyze gas exchange parameters, such as net photosynthetic rate, stomatal conductance, and transpiration; growth parameters, such as dry weight, height, and leaf area; and the anatomy of leaves and stems via root, stem, and leaf dimensions and histochemistry. The cluster divided the genotypes into six groups. The Ipiranga-01, CCN-10, and PH-16 genotypes were grouped since they presented the highest means of anatomical variables and photosynthetic parameters. The PS-1319 genotype was segregated from the others for having the lowest physiological parameter values. CCN-51 and Cepec-2002 were grouped due to their similarity only in the internal concentration of CO₂, while Ipiranga-01, CCN-10, SJ-02, and PH-16 were grouped due to having higher physiological parameters and morphoanatomical variables. The results indicated an intergenotypic variation in physiological and morphoanatomical variables, serving as a basis for the six genotype groups.

Keywords: inter-clonal comparison; phytotechnics; photosynthesis; growth analysis



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1. Introduction

Theobroma cacao L. (*Malvaceae*), popularly known as cocoa, cultivated between latitudes 20° N and 20° S, is considered one of the crops of great importance in the world economy. The cocoa bean is used worldwide as a raw material for the production of chocolate and cosmetics, hence its global economic relevance [1,2]. Although cocoa is originally from the Amazon region [3], over the centuries, it has adapted to a diverse range of environments. In this regard, its relationship with the cultivation environment and its growth are currently controlled by endogenous mechanisms, such as physiology, plant hormones, energy metabolism, nutrient absorption, cell division and expansion, and genetic regulation [1,4]. This adaptation process has given rise to varieties resulting from domestication and adaptation to specific climate conditions [5]. This process indicates the genetic diversity of these plants in response to or through tolerance to variations and fluctuations in microclimatic parameters [6–10].

Cocoa tree genotypes present high genotypic variability [11–13] and respond differently to different types of abiotic stress, like changes in temperature, humidity, light,

and water availability [6,14–17]. Therefore, it is important to identify the characteristics that determine this range of behavior. Studies have identified the different responses of these genotypes to destructive diseases, such as witches' broom disease, caused by the basidiomycete *Moniliophthora perniciosa* [18–20], black pod rot, caused by a species complex of *Phytophthora* spp. [21], environmental conditions such as the availability of solar radiation [22–30], plant–water relationships [1,15,16,25–27], and environmental carbon dioxide supply [31].

The cocoa tree has great genetic variability in relation to morphological and physiological characteristics [11–13], and the details of the adjustments of its physiological and growth processes in response to the environment are not well understood. Gas exchange and plant–water relationship analyses have identified variables that explain different behaviors in relation to these factors. The ecophysiological responses of Criollo cacao tree cultivars subjected to water deficit were studied, and it was identified that cultivars with greater osmotic adjustment showed higher survival rates [14]. In another study, total dry weight and relative growth rate were considered to identify cocoa genotypes in terms of tolerance to water deficit, classifying them as tolerant, moderately tolerant, and sensitive to lack of water [16].

When plants interact with certain environmental conditions, their metabolic balance can be altered, which impacts their morphogenesis. In this regard, the architectural pattern of the tissue in different organs can also explain their physiological behavior [28]. However, few morphoanatomical studies have been conducted on the connection between the growth, physiology, and anatomy of cacao tree genotypes grown in Brazil, especially regarding the characterization of commercial genotypes. Most studies explore this factor from the perspective of biotic [15] and abiotic stress [23,29], and few studies anatomically compare the varieties to establish differences and possible relationships with characteristics of resistance to these stressors. Despite being extensively studied from the perspective of growth, anatomy, and physiology, these genotypes have not yet been tested comparatively in the same environment.

Different environments present different physiological effects due to the modifications of the morphoanatomical dimensions of the leaf caused by the adaptation of the plant [31,32]. In the study of cocoa trees, the data from the analysis of growth and leaf anatomy are correlated with photosynthetic parameters, such as photosynthetic rate, transpiration, and water use efficiency [33,34]. In addition, microenvironmental conditions in cultivation systems are related to the metabolism of cocoa trees and their leaf characteristics [8], making knowledge about the characteristics inherent to each crucial cocoa genotype. Thus, it is possible to identify whether the adaptations are of environmental or endogenous origin, which is important when making decisions within the cultivation environment. The plasticity of morphological characteristics indicates that intergenotypic differences can be identified through phenotypic studies, can be interpreted as a response to microclimatic conditions [10], and are directly related to physiological processes [34].

Considering that cocoa production is directly controlled by physiological factors [1] and that plant tissues are involved, this fact is of great relevance when choosing genotypes [8,16,33,34]. Taking into account the interaction between environmental conditions and the characteristics of this organ and how it can be used to evaluate the effect of these attributes on physiological processes [35], it is essential to investigate these characteristics, as little is known about the interactions between the morphoanatomy and physiology inherent to each genotype.

The hypothesis tested was that morphoanatomical and physiological characteristic, in addition to differentiating genotypes, can be used to indicate which genotypes are more or less prone to adaptations to environmental conditions. The objective of this study was to describe morphoanatomical and physiological patterns that can differentiate the seven cocoa genotypes, evaluated under the same conditions of temperature, humidity, and light in a comparative way. To better understand the high plasticity presented by these genotypes for practical applications in future research in the area of phytotechnics,

improvement of techniques and cultures, propagation, and pre-selection of cocoa genotypes must be achieved.

2. Materials and Methods

2.1. Plant Material and Cultivation Conditions

Seven genotypes of *T. cacao* L., namely Ipiranga-01, CCN-10, CCN-51, SJ-02, Cepec-2002, PH-16, and PS-1319, currently recommended by the Cocoa Research Center (CEPEC/CEPLAC; Bahia, Brazil) due to high agronomic performance [31] and drought tolerance [16], were evaluated and grown in a greenhouse between 20 September and 30 October 2019 (40 days), on the campus of the Universidade Estadual de Santa Cruz (State University of Santa Cruz) (UESC), Ilhéus, BA, Brazil (14°47′00″ S, 39°02′00″ W). The climate in the region is tropical equatorial (Af), according to Köppen–Geiger classification [36]. The experiment was monitored using a data logger (Onset Computer-HOBO U-12-012, Bourne, MA, USA), recording average values of temperature (°C), relative humidity (RU%), and photosynthetically active radiation (PAR, photons m⁻² s⁻¹). September showed temperatures between 24 and 29 °C, with an average of 25.8 °C, relative humidity between 71 and 90 RU%, with an average of 82.5 RU%, and PAR between 289.96 and 794.5 photons m⁻² s⁻¹, with an average of 516.64 photons m⁻² s⁻¹. October showed temperatures between 25 and 30 °C, with an average of 26.7 °C, relative humidity between 76 and 88 RU%, with an average of 79.5 RU%, and PAR between 132.5 and 727.9 photons m⁻² s⁻¹, with an average of 440.36 photons m⁻² s⁻¹ (Figure 1).

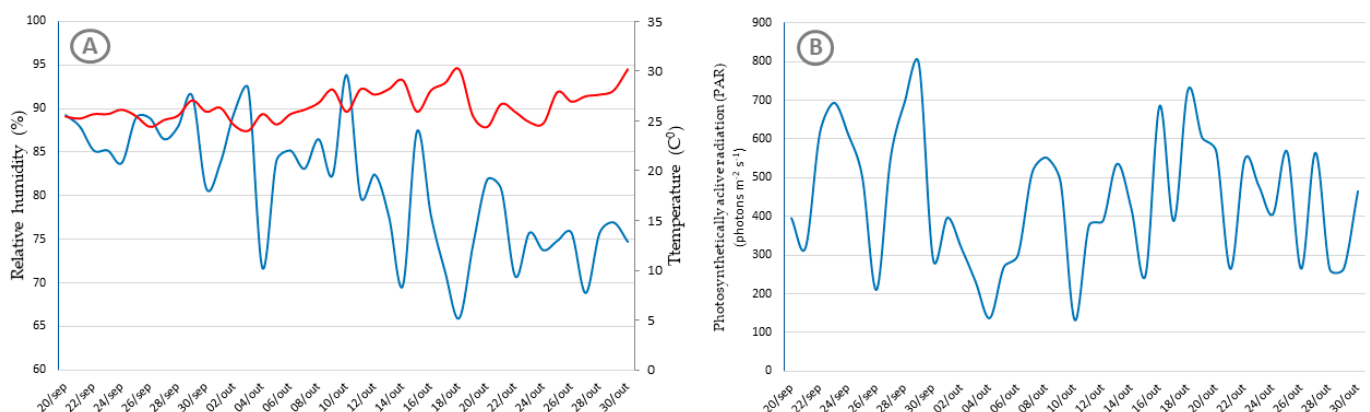


Figure 1. Environmental conditions during the experiment period: (A) temperature (°C, red line) and relative humidity (% , blue line); (B) photosynthetically active radiation (photons m⁻² s⁻¹).

Seedlings produced by the cutting method at the age of three months were acquired from the Biofábrica da Bahia, Ilhéus, BA. The plants were transplanted into pots containing 4.3 kg of air-dried soil and initially acclimatized for 10 days in a greenhouse, after which they remained cultivated under similar environmental conditions for the 40 days of the experiment (50 days after planting). During the experiment, the pH of the soil was corrected with 2 g dm⁻³ of dolomitic limestone, followed by fertilization with 5 g dm⁻³ triple superphosphate at the time of transplantation and with 33 mg dm⁻³ of urea and 25 mg dm⁻³ of potassium nitrate on the topsoil after 15 days, as recommended by Souza Junior [37] for cocoa trees.

Irrigation was defined by previously analyzing pot capacity (PC), determined using the gravimetric method adapted from Souza et al. [38] and Casaroli and Lier [39], with moisture maintained at 90% of PC.

The experiment began 10 days after planting and lasted 40 days. Under similar environmental conditions, samples for growth, anatomy, and histochemistry analysis were collected at the beginning (T0) and at the end of the experiment (T1) in the seven genotypes.

2.2. Gas Exchange

Data were obtained individually for each genotype in the one leaf of the third internode from the apex to the base, and measurements took place between 8 a.m. and 11 a.m. and did not exceed 60 min. The net photosynthetic rate (A_n ; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), the transpiration rate (E ; $\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), the intercellular CO_2 concentration (C_i ; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), and the stomatal water vapor conductance (g_s ; $\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) were obtained using infrared gas analysis LI 6400XT (Li-Cor, Lincoln, NE, USA) under artificial photosynthetically active radiation (PAR) of $1000 \text{ photons m}^{-2} \text{ s}^{-1}$ at leaf level, atmospheric CO_2 concentration of $400 \mu\text{mol mol}^{-1}$, and block temperature set at 28°C [40]. With this, the instantaneous water use efficiency (A_n/E), the intrinsic water use efficiency (A_n/g_s), the carboxylation efficiency (A_n/C_i), the ratio between internal and external concentration of CO_2 (C_i/C_a), and photosynthesis per unit of mass (A_n/SLW) were calculated.

2.3. Growth Analysis

At the end of the experiment (T_1), the plants were collected to determine the dry weights of the RDW (root dry weight; g), SDW (stem dry weight; g), and LDW (leaf dry weight; g) using a forced air oven (70°C) until constant mass. The basal diameter of the stem was measured at substrate level with a digital caliper (Mitutoyo, Kawasaki, Japan, 200 mm) with 0.01 resolution, and height was measured using a ruler. Total LA (leaf area; cm^2) was measured with an automatic meter (LI-3100, Li-Cor, Lincoln, NE, USA). Based on the SDW, RDW, and LDW, growth analysis was evaluated using the variables RWR (root weight ratio; $\text{g g}^{-1}\text{day}^{-1}$) (RDW/TDW); SWR (stem weight ratio; g g^{-1}) (SDW/TDW); and LWR (leaf weight ratio; g g^{-1}) (LDW/TDW), in addition to SLA (specific leaf area; $\text{cm}^2 \text{ g}^{-1}$) (LA/LDW); RGR (relative growth rate; $\text{g g}^{-1} \text{ day}^{-1}$) ($\text{Ln TDW}_1 - \text{Ln TDW}_0 / T_1 - T_0$); NAR (net assimilation rate; $\text{g m}^{-2} \text{ day}^{-1}$) ($\text{TDW}_1 - \text{TDW}_0 / \text{LA}_1 - \text{LA}_0 / \text{Ln LA}_1 - \text{Ln LA}_0 / T_1 - T_0$); R: AP (aerial part and root ratio; g g^{-1}) ($\text{SDW} + \text{LDW} / \text{RDW}$); LAR (leaf area ratio; $\text{cm}^2 \text{ g}^{-1}$) (LA/TDW); SLW (and specific leaf weight; $\text{g}^{-1} \text{ m}^{-2}$) (LWR/LA), according to Hunt [41].

2.4. Anatomical Characterization

Segments of the root, stem, and leaf collected at the start (T_0) and end (T_1) of the experiment were fixed in a solution containing formaldehyde, acetic acid, and 70% ethanol, 1:1:18 v/v (FAA₇₀) for 48 h and subsequently stored in 70% ethanol [42]. Adventitious samples were collected from the root tips, around 5 mm from the cap, the stem, the third internode from the apex to the base, and the middle third region of completely expanded leaves on the third node for anatomical and histochemical analysis.

According to the manufacturer's recommendations, the materials were fixed in methacrylate resin (Historesin, Leica Instruments, Heidelberg, Germany). Longitudinal and transverse sections around $5 \mu\text{m}$ thick were obtained using rotation microtome (RM 2235, Leica Microsystems, Deerfield, IL, USA). The obtained sections were stained with toluidine blue pH 4.5 [43]. Measurements were taken of the thicknesses of the epidermal cells of the adaxial and abaxial surfaces, the palisade parenchyma, the spongy parenchyma, leaf mesophyll and stem cortex, and medulla and stem diameter in cross-section using digital photomicrographs with Leica Application Suite V4.1. Four replications were performed and analyzed for each genotype.

2.5. Histochemical Analysis

Histochemical analysis was performed on fragments of plant organs from the genotypes collected 40 days after the start of the experiment in fresh matter and stored in a neutral-buffered formalin solution [44]. Transverse sections of the root, stem, and leaves were mounted on histological slides and subjected to formalin-ferrous sulfate (FFS) reagents [42] to reveal phenolic compounds, Lugol's solution for starch [42], ruthenium red solution for mucilage [45], and toluidine blue aqueous solution pH 4.5 for lignin [43].

2.6. Stomatal Density

Stomatal density per mm^{-2} was determined from digital photomicrographs obtained from epidermal impressions of the adaxial and abaxial surfaces, from the region of the middle third of leaves located on the third node, using instant glue [45]. All microscopy analyses and documentation were carried out using a photomicroscope (DM2500, Leica Microsystem; Wetzlar, Germany) with a digital image capture system (DFC295, Leica Microsystem, Wetzlar, Germany). All measurements were performed using photomicrographs with the software *Leica Application Suite V4*.

2.7. Statistical Analysis

The design was randomized (CRD) using seven genotypes (treatments) with four replications for all variables and parameters evaluated. All gas exchange, growth, and anatomy data were based on their normal distribution using the Shapiro–Wilk test with a significance of $p < 0.05$. Analysis of variance (ANOVA), F test, and means discriminated by the Scott–Knott cluster analysis method for grouping means were performed with $p < 0.05$.

Data were subjected to principal component analysis (PCA) to assess clarity between morphoanatomical and physiological data. A dendrogram was developed from multivariate cluster analysis with a model using the grouping and distance method, with Bray–Curtis similarity index and unweighted pair group method with arithmetic mean (UPGMA), using the mean values of the variables' significant quantitative results in the F Test. All parametric statistical analyses were performed using the Tinn-R statistical software (Auckland, New Zealand, version 4.3.1) [46].

3. Results

The experiment results indicate an intergenotypic difference between the genotypes in the physiological, growth, and anatomical parameters evaluated. Significant differences ($p < 0.001$) were observed between genotypes for A_a , g_s , E , C_i , and A_a/g_s (Table 1). The variation in the means of gas exchange parameters was around 60% between genotypes for A_a and 40% for g_s , with emphasis on genotypes such as CCN-51, Cepec-2002, and PS-1319, with lower g_s [0.02; 0.03 and 0.02 $\mu\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$] values and greater water use efficiency since they maintained similar A_a .

There was a difference ($p < 0.001$) between genotypes for growth variables (Table 2) and parameters (Table 3). The root dry weight (RDW) was higher [5.0 g] in the CCN-10 genotype, followed by SJ-02 and Cepec-2002 genotypes. In the genotype PS-1319, the leaf area ratio (LAR) was higher [162.3 cm cm^{-1}], with greater accumulation of dry weight in the leaf compared to the others. In the genotypes Ipiranga-01, CCN-10, and Cepec-2002, SJ-02 intermediate values were found, and Ipiranga-01, CCN-51, PH-16, and PS-1319 genotypes showed lower RDW values [2.6 g; 1.6 g; 1.6 g; 2.2 g, respectively]. In the genotypes CCN-51 and PS-1319, higher stem weight ratio (SWR) values [0.40 g g^{-1}] were observed, with greater dry weight accumulation in the stem than in the other genotypes studied.

However, LDW was higher [3.6 g] in the genotype Ipiranga-01, which had lower RDW [2.6 g] values. In the genotypes Ipiranga-01, CCN-10, and PS-1319, greater LA [837.6 cm^2 ; 805.0 cm^2 ; 950.4 cm^2] and differences in the number of leaves were observed, while CCN-10 exhibited similar A_a . In PH-16, high values of SLW [0.005 g cm^{-2}] were found, higher R:AP [0.82 g g^{-1}] was observed in the CCN-10 genotype, as well as a higher [11.7 g] TDW, with a greater number of leaves and higher [15.2] leaf area [805.0 cm^2] and RDW [5.0 g], although they were not related to A_a .

The genotype CCN-10 had greater NAR [0.27 $\text{g cm}^{-2} \text{day}^{-1}$] than the other genotypes studied during the experiment period, and the RGR was higher [21.37 and 21.43 $\text{mg g}^{-1} \text{day}^{-1}$, respectively] for the genotypes Ipiranga-01 and CCN-10. These genotypes accumulated greater total dry weight during the experiment than others.

All the genotypes had the same configuration of the lining, filling, and vascular tissues of the root, stem, and leaf, albeit with significant variation ($p < 0.05$) in the dimensions of these tissues (Table 4, Figures 1–3).

Table 1. Mean values followed by the standard deviation of gas exchange parameters of *Theobroma cacao* L. genotypes.

Genotypes	Variables								
	A_a	g_s	E	Ci	Ci/Ca	A_a/g_s	A_a/E	A_a/Ci	A_m
Ipiranga-01	4.16 ± 0.230 a	0.04 ± 0.004 b	1.12 ± 0.080 b	212.29 ± 8.40 a	0.52 ± 0.020 b	105.00 ± 4.200 a	3.7 ± 0.180 b	0.02 ± 0.003 a	984.25 ± 120 b
CCN-10	2.63 ± 0.220 b	0.04 ± 0.009 b	0.73 ± 0.040 c	219.12 ± 11.70 a	0.58 ± 0.060 b	98.9 ± 6.34 b	3.6 ± 0.260 b	0.01 ± 0.00 b	687.67 ± 170 b
CCN-51	3.27 ± 0.3 9 b	0.02 ± 0.003 d	0.79 ± 0.150 c	202.68 ± 41.60 a	0.51 ± 0.11 b	109.63 ± 6.67 a	4.2 ± 0.570 a	0.02 ± 0.001 b	945.62 ± 310 b
SJ-02	3.35 ± 0.55 b	0.05 ± 0.002 a	1.37 ± 0.050 a	262.76 ± 24.40 a	0.70 ± 0.10 a	69.44 ± 6.84 d	2.4 ± 0.200 b	0.01 ± 0.001 b	742.85 ± 280 b
Cepec-2002	3.30 ± 0.78 b	0.03 ± 0.006 c	0.67 ± 0.060 d	236.42 ± 17.40 a	0.61 ± 0.05 a	99.81 ± 6.49 b	4.9 ± 0.230 a	0.01 ± 0.003 b	789.2 ± 120 b
PH-16	3.34 ± 0.15 b	0.03 ± 0.003 c	0.85 ± 0.070 c	220.75 ± 38.70 a	0.52 ± 0.04 b	89.70 ± 18.03 c	3.9 ± 0.760 b	0.01 ± 0.003 b	689.0 ± 120 b
PS-13.19	3.05 ± 0.27 b	0.02 ± 0.004 d	0.58 ± 0.090 d	149.64 ± 33.70 b	0.52 ± 0.05 b	119.75 ± 6.38 a	5.3 ± 0.270 a	0.02 ± 0.005 a	1322.0 ± 200 a

Values followed by the same letter on the same line do not differ significantly according to the Scott–Knott test with $p \leq 0.05$. Legend: A_a (net photosynthetic rate— $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); g_s (stomatal conductance— $\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$); E (transpiration— $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$); Ci (internal concentration of CO_2 — $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); Ci/Ca (ratio between internal and external concentration of CO_2); A/g_s (intrinsic water use efficiency); A_a/E (instantaneous water use efficiency) A_a/Ci (instantaneous carboxylation efficiency); and A_m (photosynthesis per unit of mass: A_a/SLW).

Table 2. Mean values followed by the standard deviation of growth variables for the *Theobroma cacao* L. genotypes.

Genotypes	Variables								
	RDW	SDW	LDW	TDW	SD	RL	Height	LN	LA
Ipiranga-01	2.6 ± 0.33 c	2.6 ± 0.61 a	3.6 ± 0.63 a	9.0 ± 0.62 b	0.70 ± 0.10 a	38.0 ± 9.14 a	59.0 ± 8.70 a	12.0 ± 3.26 b	837.6 ± 73 a
CCN-10	5.0 ± 0.83 a	2.9 ± 0.47 a	3.3 ± 0.50 a	11.7 ± 1.52 a	0.71 ± 0.01 a	40.5 ± 7.10 a	43.4 ± 4.30 b	15.2 ± 2.87 a	805.0 ± 130 a
CCN-51	1.6 ± 0.31 c	2.3 ± 0.45 a	1.2 ± 0.23 c	7.1 ± 1.0 c	0.77 ± 0.08 a	30.2 ± 6.73 a	35.0 ± 1.76 c	7.7 ± 0.50 c	336.4 ± 92 c
SJ-02	3.4 ± 0.99 b	2.7 ± 0.37 a	3.3 ± 1.0 a	9.4 ± 0.73 b	0.70 ± 0.21 a	48.8 ± 9.70 a	44.0 ± 2.95 b	9.2 ± 0.50 c	670.4 ± 185 b
Cepec-2002	3.5 ± 0.73 b	2.8 ± 0.45 a	3.0 ± 0.57 a	9.2 ± 0.41 b	0.70 ± 0.09 a	31.5 ± 1.31 a	37.0 ± 2.90 c	11.0 ± 1.63 b	679.9 ± 32 b
PH-16	2.8 ± 0.07 c	2.3 ± 0.30 a	2.8 ± 0.26 a	8.0 ± 1.1 c	0.73 ± 0.13 a	37.5 ± 3.22 a	39.9 ± 1.50 b	9.2 ± 1.25 c	572.4 ± 57 b
PS-13.19	2.2 ± 0.20 c	2.8 ± 0.50 a	2.1 ± 0.44 b	7.9 ± 0.57 c	0.70 ± 0.13 a	37.0 ± 6.84 a	42.5 ± 5.16 b	8.0 ± 0.81 c	910.4 ± 108 a

Values followed by the same letter on the same line do not differ significantly according to the Scott–Knott test with $p \leq 0.05$. Legend: RDW (root dry weight, g); SDW (stem dry weight, g); LDW (leaf dry weight, g); TDW (total dry weight, g); SD (stem diameter, cm); RL (root length, cm); height (plant height, cm); LN (leaf number); LA (leaf area, cm^2).

Table 3. Mean values followed by the standard deviation of growth parameters for the *Theobroma cacao* L. genotypes.

Genotypes	Parameters								
	RWR	SWR	LWR	LAR	R:AP	SLA	SLW	RGR	NAR
Ipiranga-01	0.32 ± 0.06 a	0.32 ± 0.02 b	0.36 ± 0.08 a	92.9 ± 11.5 b	0.43 ± 0.04 c	236.87 ± 0.00 a	0.004 ± 0.00 a	20.37 ± 1.70 a	0.21 ± 0.03 b
CCN-10	0.43 ± 0.06 a	0.25 ± 0.06 b	0.30 ± 0.07 a	67.5 ± 13.20 b	0.82 ± 0.18 a	263.7 ± 0.00 a	0.004 ± 0.00 a	21.43 ± 3.20 a	0.27 ± 0.02 a
CCN-51	0.37 ± 0.03 a	0.40 ± 0.08 a	0.23 ± 0.04 a	63.2 ± 3.60 b	0.46 ± 0.07 c	289.10 ± 0.00 a	0.003 ± 0.00 b	12.43 ± 3.70 b	0.20 ± 0.08 b
SJ-02	0.36 ± 0.03 a	0.30 ± 0.04 b	0.35 ± 0.04 a	72.8 ± 15.8 b	0.56 ± 0.06 b	214.2 ± 0.00 a	0.005 ± 0.00 a	12.24 ± 1.90 b	0.13 ± 0.04 c
Cepec-2002	0.40 ± 0.06 a	0.31 ± 0.04 b	0.33 ± 0.03 a	80.6 ± 23.60 b	0.60 ± 0.06 b	234.8 ± 0.00 a	0.004 ± 0.00 a	9.03 ± 1.10 b	0.13 ± 0.01 c
PH-16	0.35 ± 0.06 a	0.30 ± 0.03 b	0.35 ± 0.05 a	71.7 ± 6.80 b	0.55 ± 0.05 b	205.8 ± 0.00 a	0.005 ± 0.00 a	5.45 ± 3.60 c	0.06 ± 0.04 d
PS-13.19	0.32 ± 0.05 a	0.40 ± 0.03 a	0.31 ± 0.08 a	162.2 ± 42.0 a	0.46 ± 0.08 c	433.2 ± 0.00 b	0.002 ± 0.00 b	2.66 ± 1.70 c	0.03 ± 0.03 d

Values followed by the same letter on the same line do not differ significantly according to the Scott–Knott test with $p \leq 0.05$. Legend: RWR (root weight ratio; g g⁻¹); SWR (stem weight ratio; g g⁻¹); LWR (leaf weight ratio; g g⁻¹); LAR (leaf area ratio; cm cm⁻¹); R:AP (root and aerial part ratio; g g⁻¹); SLA (specific leaf area; cm⁻² g⁻¹); SLW (specific leaf weight; g cm⁻²); RGR (relative growth rate; mg g⁻¹ day⁻¹); and NAR (net assimilation rate; g cm⁻² day⁻¹).

Table 4. Mean values followed by the standard deviation of stem and leaf anatomical variables of *Theobroma cacao* L. genotypes.

Genotypes	Variables										
	EPAD	EPAB	PP	PL	MES	PP/PL	EST	FI	X	Me	Diam
Ipiranga-01	24.0 ± 1.9 a	8.6 ± 0.6 a	33.7 ± 5.0 a	51.4 ± 8.0 a	85.2 ± 4.4 b	0.6 ± 0.1 a	1250.4 ± 23 a	169 ± 30 a	206.2 ± 40 a	1115 ± 170 a	2560 ± 150 a
CCN-10	18.7 ± 3.1 b	10.2 ± 0.9 a	35.6 ± 2.5 a	58.5 ± 5.0 a	94.2 ± 3.5 a	0.6 ± 0.1 a	862.1 ± 85 c	219 ± 40 a	285.6 ± 40 a	942 ± 100 a	2685 ± 190 a
CCN-51	23.6 ± 1.5 a	8.3 ± 1.4 a	35.7 ± 5.5 a	44.5 ± 8.0 b	80.2 ± 5.2 b	0.8 ± 0.1 a	941.3 ± 50 c	93 ± 15 b	156 ± 20 b	956 ± 240 a	2210 ± 115 b
SJ-02	16.8 ± 2.0 b	9.5 ± 1.3 a	35.4 ± 6.0 a	59.5 ± 7.5 a	95.0 ± 6.0 a	0.6 ± 0.0 a	1035.6 ± 40 b	169 ± 50 a	270 ± 60 a	1152 ± 130 a	2760 ± 120 a
Cepec-2002	18.6 ± 0.8 b	8.4 ± 0.9 a	28.4 ± 8.0 a	40.2 ± 4.0 b	68.7 ± 12.0 b	0.7 ± 0.1 a	1068.8 ± 30 b	200 ± 30 a	218.0 ± 20 a	1246 ± 70 a	2874 ± 65 a
PH-16	17.20.9 b	11.3 ± 0.6 b	33.5 ± 5.5 a	59.6 ± 5.0 a	93.1 ± 10.0 a	0.5 ± 0.0 a	917.5 ± 70 c	147 ± 20 a	232.0 ± 30 a	1158 ± 100 a	2761 ± 150 a
PS-1319	24.7 ± 2.1 a	9.6 ± 0.4 a	41.1 ± 2.5 a	52.8 ± 5.0 a	93.9 ± 4.5 a	0.8 ± 0.1 a	900.1 ± 65 c	153 ± 30 a	204.4 ± 30 a	1262 ± 100 a	2830 ± 160 a

Values followed by the same letter on the same line do not differ significantly according to the Scott–Knott test with $p \leq 0.05$. Legend: EPAD (epidermal cells of the adaxial surface, µm); EPAB (epidermal cells of the adaxial surface, µm); PP (palisade parenchyma, µm); PL (spongy parenchyma, µm); MES (leaf mesophyll, µm); PP/PL (relationship between the thickness of the parenchyma of leaves); EST (stomata density, n° mm⁻²); FI (phloem, µm) (FI); X (xylem, µm); me (medulla diameter, µm); and Diam (stem diameter, µm).

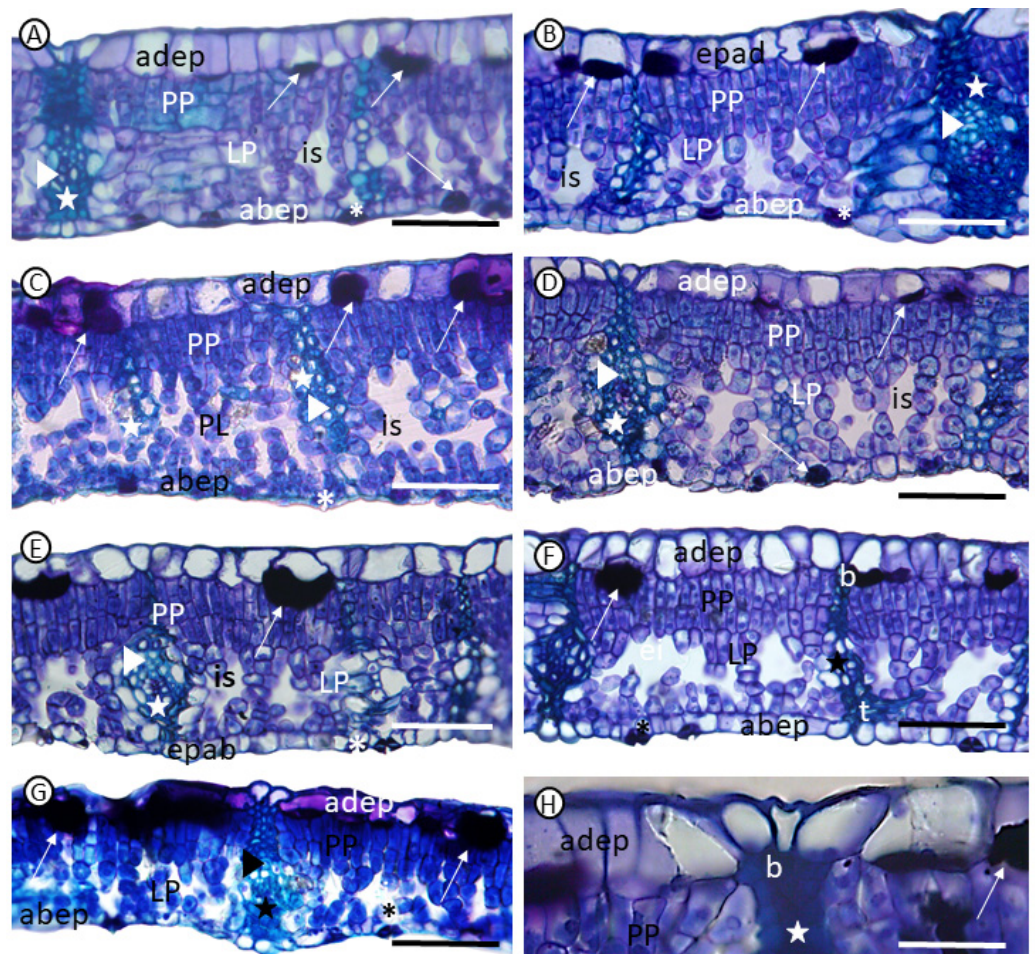


Figure 2. Cross-section of the leaf of the *Theobroma cacao* L. genotypes. (A): Ipiranga-01; (B): CCN-10; (C): CCN-51; (D): SJ-02; (E): Cepec-2002; (F): PH-16; (G): PS-1319; and (H): PS-1319. Arrow head: vascular bundle; t: bundle sheath extension; arrow head: star: vascular bundle sheath; adept: adaxial epidermal surface; abep: abaxial epidermal surface; PP: palisade parenchyma; SP: spongy parenchyma; *: stomata; is: intercellular space; arrow: mucilaginous idioblast. (A–G): bar = 50 µm; (H): bar = 20 µm.

The hypostomatic leaf exhibited a simple epidermis composed of irregular, rectangular cells covered by a fine cuticle. Although the Ipiranga-01 genotype had higher stomatal density (EST) [$1250.4 \text{ n}^0 \text{ mm}^2$] and A_a values [$4.16 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$], the SJ-02 genotype had higher E [$1.37 \text{ H}_2\text{O m}^{-2} \text{ s}^{-1}$] and g_s [$0.05 \mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$] values.

Mesophyll thickness (MES) of the genotypes CCN-51 and Cepec-2002 was lower [$80.2 \mu\text{m}$ and $68.7 \mu\text{m}$, respectively], as occurred with the spongy parenchyma (LP), which exhibited less thickness [$44.5 \mu\text{m}$ and $40.2 \mu\text{m}$] in the same genotypes in which the MES was lower. The genotypes Ipiranga-01, CCN-10, SJ-02, PH-16, and PS-1319 had higher MES [$85.2 \mu\text{m}$; $94.2 \mu\text{m}$; $95.0 \mu\text{m}$; $93.1 \mu\text{m}$ and $93.9 \mu\text{m}$, respectively] values, which may have influenced gas exchange. The LP presented evident, wide intercellular spaces in the genotypes CCN-10, CCN-51, and SJ-02, filling 54% to 64% of the mesophyll. The LP thickness values were higher [$58.5 \mu\text{m}$, $59.5 \mu\text{m}$, $59.6 \mu\text{m}$, respectively] for the CCN-10, SJ-02, and PH-16 genotypes. The genotypes CCN-51 and Cepec-2002 had a similar A_a to that of the genotypes CCN-01, SJ-02, PH-16, and PS-1319, but lower mesophyll thickness [$80.2 \mu\text{m}$; $68.7 \mu\text{m}$]. The only genotype with thick mesophyll [$95.0 \mu\text{m}$] and high g_s [$0.05 \mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$] was SJ-02.

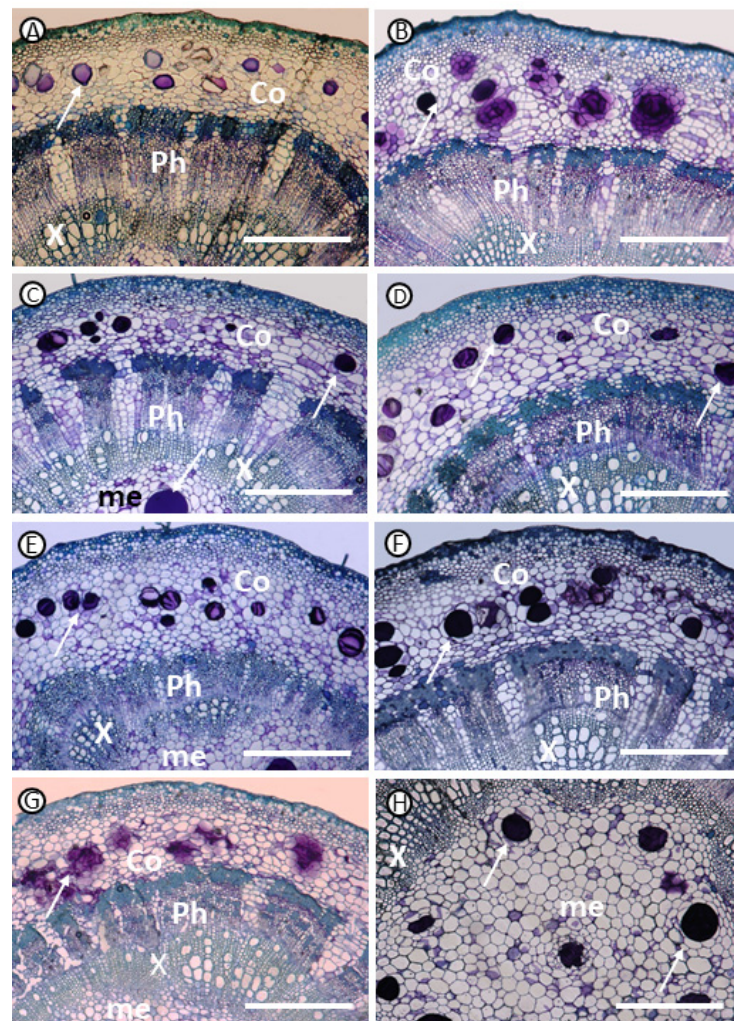


Figure 3. Cross-section of the stem of *Theobroma cacao* L. genotypes. (A): Ipiranga-01; (B): CCN-10; (C): CCN-51; (D): SJ-02; (E): Cepec-2002; (F): PH-16; (G): PS-1319; and (H): PS-1319. Co: cortex; X: xylem; Ph: phloem; me: medulla; arrow: mucilage secretory cavity. (A–G): bar = 200 μ m; (H): bar = 100 μ m.

In the mesophyll of all genotypes, the vascular bundles were surrounded by a sheath of parenchymal cells extending to the epidermis' abaxial and adaxial surfaces. The PP had three to four cellular strata (Figure 2), varying between genotypes, with four in the SJ-02 genotype and three in the other genotypes. The stems of all the analyzed cocoa tree genotypes had a secondary growth stage (Figure 3A–G).

However, in most cases, the primary surface system remained covered by the cuticle, with glandular and non-glandular trichomes. The stem the cortical region was constituted by a simple hypodermis, followed by layers of collenchyma and parenchymal cells. The vascular cylinder exhibited secondary phloem, xylem, and vascular cambium activity. The secondary phloem was interspersed with fiber bands with a unique pattern in the CEPEC-2002 genotype (Figure 3E). Broad parenchymal rays were observed in the secondary phloem and xylem region. Mucilage channels characteristic of the species were observed in all genotypes' cortex and parenchymal medulla (Figure 3A–H).

The adventitious roots of all the studied genotypes had a primary structure without structural distinction (Figure 4A–D) with simple epidermis and exoderm, the latter consisting of thickened wall cells. The cortex had parenchymal cells with wide intercellular spaces.

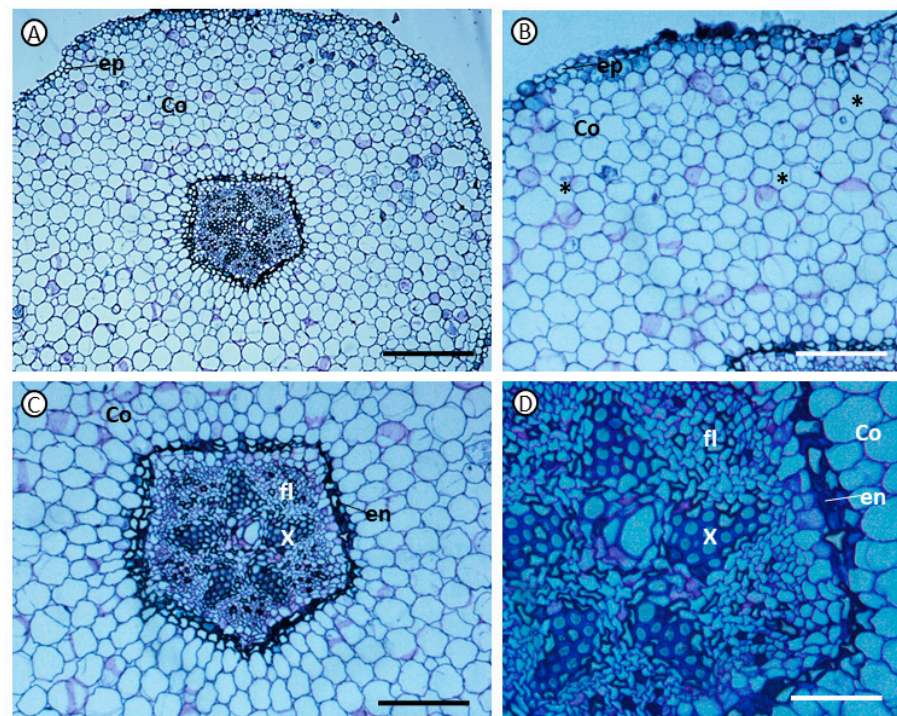


Figure 4. Cross-section of the adventitious root of the Ipiranga-01 genotype of *Theobroma cacao* L. Co: cortex; ep: epidermis; en: endoderm; asterisk: intercellular space; X: Xylem; fl: phloem. (A): bar = 200 μ m; (B,C): bar = 100 μ m; (D): bar = 100 μ m.

Endoderm rich in phenolic compounds was a common aspect of all the genotypes, as was vascular tissue with polyarch condition occupying the entire central region of the organ (Figure 4A–C). Mucilage (Figure 5A,B) was observed in the idioblasts present on the adaxial surface of the leaf epidermis, in the guard cells of the stomata (Figure 5A), and in cavities in the cortex and stem medulla of all the investigated clones.

A positive reaction was observed for the phenolic compound in the abaxial and adaxial epidermis and vascular bundle sheath in the leaf, the outer layer of the cortex and phloem region in the stem, as well as the endoderm and root phloem, thus forming a phenolic sheath around the vascular bundle (Figure 5C,D). The presence of a sheath in the leaf vascular bundle was observed in all the analyzed genotypes. Starch is present in the parenchymal cells of the coastal sheath extension of the midrib (Figure 5E), in the endoderm, in the outer cortical layers, and in the stem pith (Figure 5F). A polarized light filter revealed calcium oxalate biominerals in the form of drusen and prisms in the outer cortical region of the phloem in the stem and in the spongy parenchyma and vascular bundle sheath of the leaf.

In the interaction of the results, when considering the data related to photosynthesis and morphoanatomy in the principal component analysis coordinate system (Figure 6), two components were responsible for 74% of the data variation. The first component explains 47% and the second component 27%, which is sufficient since the eigenvalues of both components are greater than one, differing from the others. In this coordinate system of the two components, it is possible to identify the clusters associated with each genotype based on the factor loads. The values that most affected the components were MES, LP, E , g_s , and RGR in Component 1, and values of physiological parameters such as A_n and C_i in Component 2. This interaction is given among genotypes PH-16 and CCN-10 by morphoanatomical variables MES and LP and in Ipiranga-01 by photosynthetic parameters A_n , E , g_s , and C_i , and RGR growth.

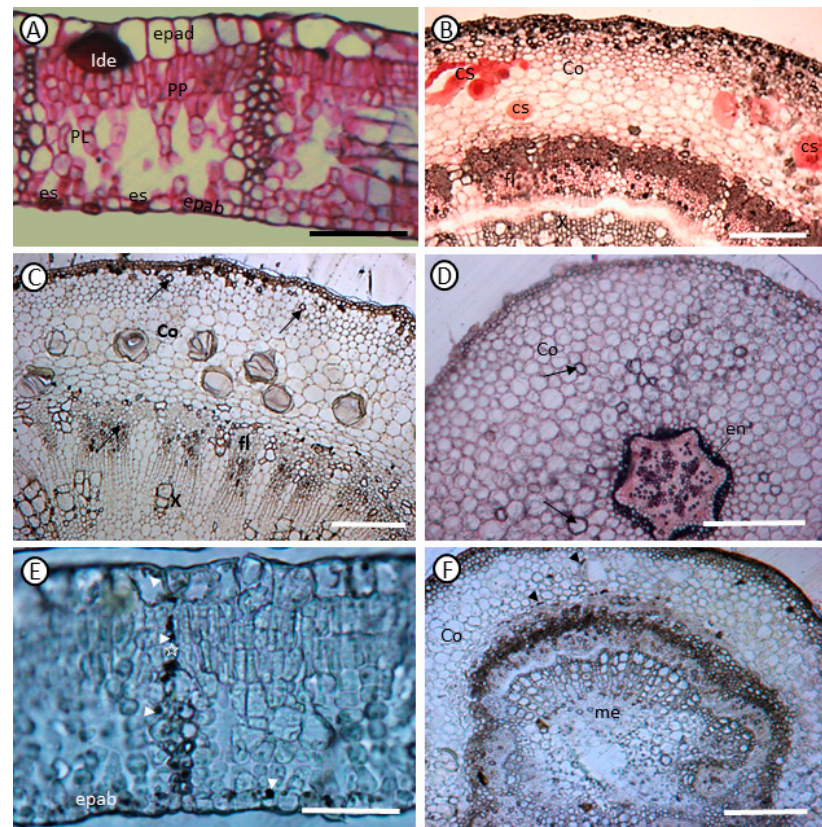


Figure 5. Histochemistry of the root, stem, and leaf of *Theobroma cacao* L. clones (cross-section). (A,B): Ruthenium red staining highlights the density of the pectic content in the epidermal idioblast, in the wall of the stomatal guard cells (A), and in the secretory channels of the cortical region of the stem (B). (C,D): Positive reaction to ferrous sulfate, highlighting the presence of phenolic idioblasts in the cortex and associated with phloem and xylem of the stem of (C) and in the cortex and endoderm of the root of (D). (E,F): Positive reaction to Lugol highlights the presence of starch in the epidermis and the sheath of the vascular bundle of the leaf (E) and in the cortical region and starch sheath (endoderm) of the stem (F). Eid: epidermal idioblast; cs: secretory cavity; arrow: phenolic compound; arrowhead: starch grains. epab: abaxial epidermal surface; Co: cortex; en: endoderm; X: xylem; fl: phloem; me: medulla; star: vascular bundle sheath; es: stomata. (A,C,D): bar = 50 μ m; (B): bar = 100 μ m; (F): bar = 200 μ m.

According to the data, the genotypes Ipiranga-01, CCN-10, PH-16, and SJ-02 had the highest morphoanatomical and physiological data values described for Component 1, while the genotypes CEPEC-2002, CCN-51, and PS-1319 had the lowest values for these characteristics. Therefore, this analysis illustrates the relationship between morphoanatomical and physiological patterns and reveals a tendency toward different genotype behaviors under similar environmental growth conditions separated into two large groups (Figure 7).

Once these two groups were established in the principal component analysis (PCA), the seven genotypes studied here were grouped by multivariate cluster analysis in a similarity dendrogram (Figure 7) using values of morphoanatomical characteristics (MES, LP, SD, and RGR) and physiological parameters (A_a , g_s , E , and C_i).

The genotypes that most closely resembled these values were Ipiranga-01, CCN-10, and PH-16, which are in the same group and showed a similarity of around 96% based on morphoanatomical and physiological characteristics (Figure 7); the others genotypes are in different groups because they have lower physiology data values, as in the case of PS-1319 in a stand-alone group with 84% similarity to the others, and low morphoanatomy and physiology values, as in the case of genotypes Cepec-2002, CCN-51, and SJ-02, with 93% similarity to the other groups.

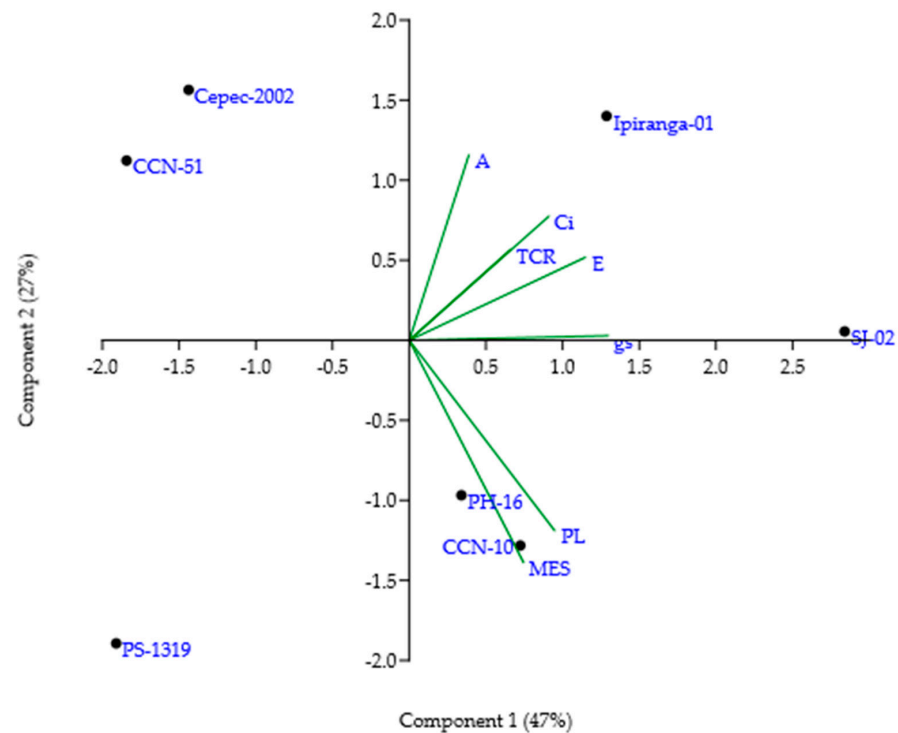


Figure 6. Principal component analysis of morphoanatomical variables—spongy parenchyma (LP), mesophyll thickness (MES), and relative growth rate (RGR). Physiological parameters—photosynthetic rate (A_n), transpiration (E) and stomatal conductivity (g_s), internal concentration of CO₂ (C_i). Seven *Theobroma cacao* L. genotypes were kept in similar growing conditions for 40 days in a greenhouse.

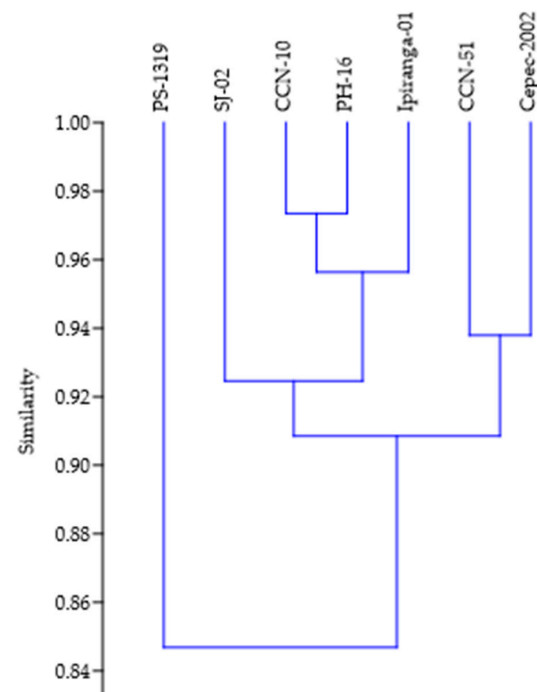


Figure 7. Dendrogram with the *Theobroma cacao* L. genotype clusters kept in similar growing conditions for 40 days in a greenhouse, obtained using cluster analysis based on the Brian–Curtis similarity index, taking into account the morphoanatomical variables mesophyll thickness (MES), spongy parenchyma (LP) thickness, stomatal density (SD), and relative growth rate (RGR), and the photosynthetic parameters photosynthetic rate (A_n), stomatal conductance (g_s), internal concentration of CO₂ (C_i), and transpiration rate (E).

Cocoa tree genotypes can be grouped based on their morphoanatomical and physiological characteristics due to the need to better understand plant genetic variability and adaptability to the environment. These factors are essential for selecting more productive, resistant varieties adapted to specific growing conditions.

4. Discussion

The genotypes CCN-51, Cepec-2002, and PS-1319 showed high A_a/g_s , which, according to Muller et al. [47], enables greater carbon assimilation during photosynthesis while also controlling transpiration, that is, greater carbon assimilation per unit of transpired water. Low g_s values in the genotypes Ipiranga-01, CCN-10, CCN-51, Cepec-2002, PH-16, and PS-1319 can have a protective effect on plants [7]. Still, this fact does not necessarily indicate that the stomata are regulating the rate of photosynthesis, but could be a possible consequence of the response to luminosity, temperature, and humidity [34], enhancing A_a/E but decreasing internal CO_2 concentration [48]; low g_s values may not be as beneficial, as this reduction causes an increase in leaf temperature by reducing the flow of water vapor [35].

The greater accumulation of mass in the roots in the CCN-51, Cepec-2002, and SJ-02 genotypes enabled greater water absorption to meet the transpiration demand [49] caused by the high g_s in the SJ-02 genotype, which reduced the efficiency of water use concerning other genotypes [50]. The greater total dry weight in the CCN-10 genotype may have been influenced by the greater number of leaves, leaf area, and root weight, although unrelated to A_a [17]. The variation in RDW, greater in the CCN-10, SJ-02, and Cepec-2002 genotypes, and A_a/E in the CCN-51 and Cepec-2002 genotypes, demonstrates that these genotypes have drought tolerance characteristics [28] and can be introduced into programs of improvement for planting in non-traditional or marginal areas to those currently cultivated [51,52]; this shows how fundamental it is to know the growth behavior, as what can be seen as an effect of lack of water may be just an expression of the endogenous phenotypic plasticity of each genotype [1,4].

Greater LA and differences in LN were observed, but CCN-10 exhibited similar A_a . The other genotypes with greater LA development, such as the PS-1319 genotype, maximized light capture [53], maintaining high A_a in Ipiranga-01, which was also found by Valladares et al. [54] and Valladares et al. [55] in studies with tropical species, indicating that increased leaf area is characteristic of shade plants [56]. Although the Ipiranga-01 genotype presented an LA similar to the CCN-10 and PS-1319 genotypes, its A_a was higher than in these genotypes.

Plants with a larger LA may have greater photosynthetic and growth potential when compared with plants with smaller LA [49,50]. Variations in leaf size are directly related to light capture [54,55] and characteristics of solar leaves that are thicker and have a smaller leaf area [57]; this morphological adaptation is a trend in drought-tolerant cocoa genotypes [28,58].

The relationship between LA and LDW (SLA) is the leaf-level trade-off between light interception and dry weight accumulation [59]. SLW is a fundamental characteristic in the growth of cocoa plants [60], and in this case, not all genotypes that presented higher LA and TDM values presented higher SLW; Ipiranga-01, SJ-02, Cepec-2002, and PH-16 presented higher SLW values than those found in the CCN-51 genotype, and only the CCN-10 genotype presented high values for LA and TDM; this fact reflects the fact that morphological differences in growth expansion or retraction tend to find a balance for the plant, since SLW is an important adaptive indicator to the environment [61,62]. Araújo et al. [23] emphasize that cocoa genotypes are affected by light intensity and may present differences in the anatomical plasticity of the leaves. High SLA values are associated with adaptation to shaded environments [63]. Stomatal density is directly related to A_a since H_2O efflux and CO_2 influx share the same path through the stomatal pore. Hence, the distribution and number of stomata influence A_a [63] and are extremely important since stomata are the main route of gas exchange, and variations in diffusive resistance can cause

changes in A_a [64], which is one of the main anatomical characteristics related to A_a [65]. Furthermore, stomatal density can be a strategy to avoid water loss [66].

Carr and Lockwood [67] report in a literature review that stomatal density may vary according to water availability in cocoa trees. In the present study, the genotype PS-1319 had the lowest stomatal density and the largest leaf area. In general, the genotypes studied here exhibited increased stomatal density associated with smaller leaf areas, as also identified by Abo-Hamed et al. [68] in their work with cocoa trees.

The spongy parenchyma (LP) is associated with diffusion and internal concentration of CO_2 ; therefore, when the LP is thicker, it can enable greater fixation of CO_2 and stomatal aperture [63]. According to Voltan et al. [69], coffee plants with greater LP thickness tend to have a higher net photosynthetic rate (A_a); mesophyll thickness is related to photosynthetic efficiency and higher water use efficiency in apple trees [70] and olive trees [71]. Moreover, these authors found greater photosynthetic efficiency in varieties with a thicker mesophyll. It can also be used as a selection criterion for genotypes of drought-resistant cocoa trees [72].

In a study by Ennajeh et al. [71] with two varieties of olive trees subjected to water deficit, greater photosynthetic efficiency was observed in the cultivar with increased thickness of the mesophyll since the palisade and spongy parenchyma control the ability of the plant to resist water deficit, which favors the fixation and diffusion of carbon dioxide. A similar situation was observed by Brodribb et al. [73,74] in eight species of angiosperms, suggesting that the proximity of the vascular bundles to the evaporative surfaces helps increase the hydraulic conductivity of the leaf and favors photosynthetic capacity. The palisade parenchyma (PP) and the spongy parenchyma are directly related to the mesophyll thickness, which can determine the stress level caused by the water deficit in cocoa trees [75]. This characteristic was reported in the study by Brooks and Guard [76] in their work with cocoa trees, highlighting that drought-tolerant genotypes reduce the dimensions of these tissues.

The only genotype with thick mesophyll and high g_s was SJ-02, indicating that leaf anatomy is associated with the CO_2 transfer effect and is critical in gas exchange [77]. Morphoanatomical variations of the leaf can affect physiological characteristics. Thus, the interaction between environmental conditions and the characteristics of this organ can be used to evaluate the effect of these attributes on physiological processes such as transpiration [35].

The hydraulic architecture of the vascular leaf tissue can determine the physiological pattern of plants since water transport is affected by low resistance of the vascular system and high resistance in the mesophyll [28]. According to Ennajeh et al. [35], varieties with thicker mesophyll exhibited greater photosynthetic efficiency in a study with olive trees. Wide cell spaces were also observed in the PH-16 and CCN-10 genotypes. According to Jones [78], plants with wide intercellular spaces tend to have a rapid stomatal aperture, thus providing greater A_a/g_s by reducing transpiration, which improves survival conditions in water-stressed environments. In the present study, anatomical characteristics were related to physiological performance for the aforementioned genotypes.

According to Jordaan and Kruger [79], the presence of mucilage may be ecologically important for the conservation of hydration in plants. In a study with three tropical tree species, Chapotin et al. [80] considered that water stored in the mucilage can serve as a buffer in short periods of water deficit, and changes in water content lead to small changes in water potential. This characteristic, associated with water retention [81] and found in *T. cacao* L., remained unchanged among the evaluated clones. Nakayama et al. [82] also found mucilage only in the stem and leaf. However, Brooks and Guard [76] also report the presence of mucilage in cavities between the parenchymal cells of the roots. The phenolic compounds can act as insecticides, antimicrobials, and signals and can attract pollinators, protect against light radiation, and serve as a constituent of plant tissues [83,84].

This characteristic controls the water dynamics in response to the external environment, serving as a protective device and transforming chemical signals into hydraulic responses [85]. However, there was no relationship between this characteristic and A_a/E

and gs. In this regard, Lynch et al. [86], in their study of four tree species, found that the plasticity of the bundle sheath extension in some of these species makes it impossible to correlate this characteristic to A_a/E , especially for *Acer saccharum*.

Reserve starch is hydrolyzed by increasing amylase activity in plants under water stress, thus accumulating soluble carbohydrates, amino acids, and organic acids, which would be used to develop and grow new tissues [87]. Almeida et al. [18] studied the anatomy of SCA-6 clones and Catongo cocoa trees infected with witches' brooms. They found hypertrophy in xylem cells and the presence of starch grains associated with the medulla and vascular bundles, suggesting that starch may be related to tolerance to the fungus.

The results found in this study suggest that different cocoa tree genotypes may have distinct morphophysiological characteristics under similar environmental conditions. This demonstrates that the performance of cocoa tree genotypes depends on the combination of numerous variables, and there is an intergenotypic difference. This fact reveals the importance of this model, as it lists certain genotypes based on these characteristics and can be used to define genotypes further; it can be used as a criterion for choosing genotypes for certain cocoa cultivation systems or selecting genotypes for propagation based on these characteristics. This work provides information on morphoanatomical and physiological patterns in these genotypes, which can be used as a basis for applied work such as drought tolerance or vegetative propagation. The model can also be used as a criterion of choice for genetic pre-breeding since phenotypic (massal) selection is usually adopted in cocoa tree enhancement [88] due to its plasticity. These morphoanatomical and physiological characteristics can help in this practice [89].

Even though consistent results were found, there were limitations to the use of complementary techniques in anatomy and physiology, such as the use of scanning electron microscopy (SEM) and transmission electron microscopy (TEM), fluorescence analysis, and hydraulic conductivity, which could further deepen knowledge in the description of individual characteristics of each genotype, making it possible to more precisely identify more adapted genotypes, with desired agronomic characteristics such as drought tolerance, efficiency in nutritional responses, and high productivity.

Future research must study cocoa trees, addressing complementary techniques and research applied in other areas such as genetics, nutrition, and productivity. This will benefit producers and cocoa farming, increasing sustainability by reducing the use of inputs and water resources and adding value to the final product.

5. Conclusions

Intergenotypic variability was identified based on anatomical, growth, and physiological characteristics, showing better performance for the genotypes Ipiranga-01 and SJ-02, mainly in the parameters net photosynthetic rate and relative growth rate, and the genotypes CCN-10 and PH-16 were more associated with anatomical characteristics. The CCN-51 and Cepec-2002 genotypes presented an inferior performance based on these characteristics, while the PS-1319 genotype was set apart from the others based on low physiological parameters. The Ipiranga-01, SJ-02, and PS-1319 genotypes generally have greater physiological changes, while the CCN-10, CCN-51, PH-16, and Cepec-2002 genotypes have greater morphoanatomical changes. Morphoanatomical and physiological changes are essential for modern cocoa farming, as they allow the development of more productive and resilient cocoa cultivars.

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References

- Muller, M.F.; Valle, R.R. Ecofisiologia do cacau. In *Ciência, Tecnologia e Manejo do Cacau*, 2nd ed.; CEPLAC: Ilhéus, BA, Brazil, 2012; pp. 31–66.
- Halim, A.N.; Abd Gani, S.S.; Zaidan, U.H.; Halmi, M.I.E.; Wahab, N.A.; Yusof, A.H.M. Potentiality of incorporating cocoa liquor in skin care cosmetics. *PalArch's J. Archaeol. Egypt/Egyptol.* **2020**, *17*, 1039–1046.
- Zarrillo, S.; Gaikwad, N.; Lanaud, C.; Powis, T.; Viot, C.; Lesur, I.; Fouet, O.; Argout, X.; Guichoux, E.; Valdez, F.; et al. The use and domestication of *Theobroma cacao* during the mid-Holocene in the upper Amazon. *Nat. Ecol. Evol.* **2018**, *2*, 1879–1888. [[CrossRef](#)] [[PubMed](#)]
- Greathouse, D.C.; Laetsch, W.M.; Phinney, B.O. The stem-growth rhythm of a tropical tree, *Theobroma cacao*. *Am. J. Bot.* **1971**, *58*, 281–286. [[CrossRef](#)]
- Yamada, M.M.; Pires, J.L.; Faleiro, F.G.; Lopes, U.V.; Macedo, M.M.; Melo, G.R.; Clement, D.; dos Santos, R.C.; Dantos, A.; Santos, R.F.; et al. Histórico das seleções de cacaueiros da série ESJOB. Ilhéus, BA, CEPLAC/CEPEC. *Bol. Técnico* **2014**, *206*, 2–16.
- Almeida, A.A.F.; Valle, R.R. Ecophysiology of the cacao tree. *Braz. J. Plant Physiol.* **2007**, *19*, 425–448. [[CrossRef](#)]
- Balasimha, D.; Rajagopal, V. Stomatal responses of cocoa to changes in climatic factors. *J. Agric. Sci.* **1988**, *58*, 213–216.
- Niether, W.; Armengot, L.; Andres, C.; Schneider, M.; Gerold, G. Shade trees and tree pruning alter throughfall and microclimate in cocoa (*Theobroma cacao* L.) production systems. *Ann. For. Sci.* **2018**, *75*, 38. [[CrossRef](#)]
- Lahive, F.; Hadley, P.; Daymond, A.J. The physiological responses of cacao to the environment and the implications for climate change resilience. A review. *Agron. Sustain. Dev.* **2019**, *39*, 5–27. [[CrossRef](#)]
- Vásquez, C.E.; Hipólito-Romero, E.; Ricaño-Rodríguez, J.; Ramos-Prado, J.M. Ecophysiological plasticity of *Theobroma cacao* L. clones in response to the structure and microclimate of agroforestry systems in Mexico. *Bot. Sci.* **2022**, *100*, 960–976. [[CrossRef](#)]
- Daymond, A.J.; Hadley, P.; Machado, R.C.R.; Ng, E. Genetic variability in partitioning to the yield component of cacao (*Theobroma cacao* L.). *HortScience* **2002**, *37*, 799–801. [[CrossRef](#)]
- Daymond, A.J.; Hadley, P.; Machado, R.C.R.; Ng, E. Canopy characteristics of contrasting clones of cacao (*Theobroma cacao*). *Exp. Agric.* **2002**, *38*, 359–367. [[CrossRef](#)]
- Daymond, A.J.; Tricker, P.J.; Hadley, P. Genotypic variation in photosynthetic and leaf traits in Cocoa. In Proceedings of the International Cocoa Research Conference, Bali, Indonésia, 16–21 November 2009.
- Araque, O.; Jaimez, R.E.; Tezara, W.; Coronel, I.; Urich, R.; Espinoza, W. Comparative photosynthesis, water relations, growth and survival rates in juvenile Criollo cacao cultivars (*Theobroma cacao*) during dry and wet seasons. *Exp. Agric.* **2012**, *48*, 513–522. [[CrossRef](#)]
- Dzandu, E.; Enu-Kwesi, L.; Markwei, C.M.; Ayeh, K.O. Screening for drought tolerance potential of nine cocoa (*Theobroma cacao* L.) genotypes from Ghana. *Heliyon* **2021**, *7*, e08389. [[CrossRef](#)] [[PubMed](#)]
- Santos, I.C.D.; Almeida, A.A.F.D.; Anheer, D.; Conceição, A.S.D.; Pirovani, C.P.; Pires, J.L.; Valle, A.A.; Baligar, V.C. Molecular, physiological and biochemical responses of *Theobroma cacao* L. genotypes to soil water deficit. *PLoS ONE* **2014**, *9*, e115746. [[CrossRef](#)] [[PubMed](#)]
- Santos, E.A.; Almeida, A.A.F.D.; Ahnert, D.; Branco, M.C.D.S.; Valle, R.R.; Baligar, V.C. Diallel analysis and growth parameters as selection tools for drought tolerance in young *Theobroma cacao* plants. *PLoS ONE* **2016**, *11*, e0160647. [[CrossRef](#)] [[PubMed](#)]
- Almeida, A.A.F.; Aguiar, M.A.G.; Araújo, I.S.; Valle, R.R. Modificações Anatômicas em dois genótipos de cacau infectados com *Crinipellis pernicioso*. *Agrotrop* **1998**, *10*, 199–202.
- Sena, K.; Alemanno, L.; Gramacho, K.P. The infection process of *Moniliophthora pernicioso* in cacao. *Plant Path* **2014**, *63*, 1272–1281. [[CrossRef](#)]
- Santos, E.C.; Pirovani, C.P.; Correa, S.C.; Micheli, F.; Gramacho, K.P. The pathogen *Moniliophthora pernicioso* promotes differential proteomic modulation of cacao genotypes with contrasting resistance to witches' broom disease. *BMC Plant Biol.* **2020**, *20*, 1. [[CrossRef](#)]
- Guiltinan, M.J.; Verica, J.; Zhang, D.; Figueira, A. Genomics of *Theobroma cacao*, “the Food of the Gods”. In *Genomics of Tropical Crop Plants. Plant Genetics and Genomics: Crops and Models*, 1st ed.; Moore, P.H., Ming, R., Eds.; Springer: New York, NY, USA, 2008; pp. 145–170.
- Costa, L.C.B.; Almeida, A.A.F.; Valle, R.R. Crescimento, teor de clorofila e estrutura anatômica em plântulas de *Theobroma cacao* submetidas a diferentes irradiâncias e doses de nitrogênio. *Agrotropica* **1998**, *10*, 21–30.

23. Araújo, R.P.; Almeida AA, F.; Barroso, J.P.; Oliveira, R.A.; Gomes, F.P.; Ahnert, D.; Baligar, V. Molecular and morphophysiological responses cocoa leaves with different concentrations of anthocyanin to variations in light levels. *Sci. Hortic.* **2017**, *224*, 188–197. [CrossRef]
24. Baligar, V.C.; Elson, M.K.; Almeida AA, F.; Araujo, Q.R.; Ahnert, D.; He, Z. The impact of carbon dioxide concentrations and low to adequate photosynthetic photon flux density on growth, physiology and nutrient use efficiency of juvenile cacao genotypes. *Agronomy* **2021**, *11*, 397. [CrossRef]
25. Almeida, A.A.F.; Brito, R.C.T.; Aguilar, M.A.G.; Valle, R.R. Aspectos das relações hídricas de clones de *Theobroma cacao* L. *Agrotrópica* **2002**, *14*, 35–44.
26. Bertolde, F.Z.; Almeida, A.A.F.; Pirovani, C.P. Analysis of gene expression and proteomic profiles of clonal genotypes from *Theobroma cacao* subjected to soil flooding. *PLoS ONE* **2014**, *9*, e108705. [CrossRef] [PubMed]
27. Rehem, B.C.; Almeida AA, F.; Mielke, M.S.; Gomes, F.P.; Valle, R.R. Photosynthetic and growth responses of *Theobroma cacao* L. clones to waterlogging. *J. Trop. Agric.* **2010**, *48*, 17–22.
28. Cochard, H.; Nardini, A.; Coll, L. Hydraulic architecture of leaf blades: Where is the main resistance? *Plant Cell Environ.* **2004**, *27*, 1257–1267. [CrossRef]
29. Castro, A.V.; DEAlmeida, A.A.F.; Pirovani, C.P.; Reis, G.S.; Almeida, N.M.; Mangabeira, P.A. Morphological, biochemical, molecular and ultrastructural changes induced by Cd toxicity in seedlings of *Theobroma cacao* L. *Ecotoxic. Environ. Saf.* **2015**, *115*, 174–186. [CrossRef]
30. Monteiro, R.W.; Ahnert, D. Melhoramento Genético do Cacaueiro. In *Ciência, Tecnologia e Manejo do Cacaueiro*, 2nd ed.; Valle, R.R., Ed.; CEPLAC: Ilhéus, BA, Brazil, 2012; pp. 11–29.
31. Bote, A.D.; Struik, P.C. Effects of shade on growth, production and quality of coffee (*Coffea arabica*) in Ethiopia. *J. Hortic. For.* **2011**, *3*, 336–341.
32. Lin, B.B. The role of agroforestry in reducing water loss through soil evaporation and crop transpiration in Coffee agroecosystems. *Agr. For. Meteorol.* **2010**, *150*, 510–518. [CrossRef]
33. Daymond, A.J.; Tricker, P.J.; Hadley, P. Genotypic variation in photosynthesis in cacao is correlated with stomatal conductance and leaf nitrogen. *Biol. Plant* **2011**, *55*, 99–104. [CrossRef]
34. Avila-Lovera, E.; Coronel, I.; Jaimez, R.; Urich, R.; Pereyra, G.; Araque, O.; Chacon, I.; Tezara, W. Ecophysiological traits of adult trees of Criollo cocoa cultivars (*Theobroma cacao* L.) from a germplasm bank in Venezuela. *Exp. Agric.* **2016**, *52*, 137–153. [CrossRef]
35. Monteiro, M.V.; Blanuša, T.; Verhoef, A.; Hadley, P.; Cameron, R.W. Relative importance of transpiration rate and leaf morphological traits for the regulation of leaf temperature. *Aust. J. of Bot.* **2016**, *64*, 32–44. [CrossRef]
36. Peel, M.C.; Finlayson, B.L.; McMahon, T.A. Updated world map of the Köppen-Geiger climate classification. *Hydr. Earth Syst. Sci.* **2007**, *11*, 1633–1644. [CrossRef]
37. Souza Júnior, J.O.; Sodré, G.A.; Neves, J.C.L. Fertilidade do solo, correção de acidez e recomendação de adubação para o cacaueiro. In *Cacau, Cultivo, Pesquisa e Inovação*, 1st ed.; Souza Júnior, J.O., Ed.; Editus: Ilhéus, BA, Brazil, 2018; pp. 13–33.
38. Souza, C.C.D.; Oliveira, F.A.D.; Silva, I.D.F.D.; Amorim Neto, M.D.S. Avaliação de métodos de determinação de água disponível e manejo da irrigação em terra roxa sob cultivo de algodoeiro herbáceo. *Rev. Bras. Eng. Agrícola Ambient.* **2000**, *4*, 338–342. [CrossRef]
39. Casaroli, D.; Jong Van Lier, Q.D. Critérios para determinação da capacidade de vaso. *Rev. Bras. Ciência Solo* **2008**, *32*, 59–66. [CrossRef]
40. Von Caemmerer, S.; Farquhar, G.D. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* **1981**, *153*, 376–387. [CrossRef] [PubMed]
41. Hunt, R. *Basic Growth Analysis*; Unwin Hyman: London, UK, 1990; pp. 98–112.
42. Johansen, D.A. *Plant Microtechnique*, 1st ed.; McGraw-Hill: New York, NY, USA, 1940; pp. 521–523.
43. O'Brien, T.P.; McCully, M.E. *The Study of Plant Structure. Principles and Selected Methods*, 1st ed.; Termarcaphi Pty. Ltd.: Melbourne, Australia, 1981; pp. 333–357.
44. Pizzolatto, P.; Lillie, R.D. Mayer's tannic acid-ferrie chloride stain for mucins. *J. Histochem. Cytochem.* **1973**, *21*, 56–64. [CrossRef] [PubMed]
45. Segatto, F.B.; Bisognin, D.A.; Benedetti, M.; Costa, L.C.D.; Rampelotto, M.V.; Nicoloso, F.T. A technique for the anatomical study of potato leaf epidermis. *Ciência Rural* **2004**, *34*, 1597–1601. [CrossRef]
46. CRAN—Comprehensive R Archive Network. Available online: <https://cran.r-project.org/> (accessed on 2 January 2020).
47. Müller, M.W.; Serrano-Minar, P.; Biehl, B. Photosynthetic characteristics during development of leaves from *Theobroma cacao* L. *Physiol. Plant* **1992**, *853*, 105–599.
48. Apshara, S.E.; Rajesh, M.K.; Balasimha, D. Assessment of morphological, physiological and molecular characteristics of cocoa accessions from Central and South America in relation to drought tolerance. *J. Plant Crop.* **2013**, *41*, 389–397.
49. Poorter, L. Growth responses of 15 rain-forest tree species to a light gradient: The relative importance of morphological and physiological traits. *Funct. Ecol.* **1999**, *13*, 396–410. [CrossRef]
50. Poorter, H.; Evans, J.R. Photosynthetic nitrogen-use efficiency of species that differ inherently in specific leaf area. *Oecologia* **1998**, *116*, 26–37. [CrossRef]
51. Jegadeeswari, V.; Arunkumar, K.; Santhini, B.G. Root morphology and root characters of different cocoa (*Theobroma cacao* L.) types at 100 and 50 percent field capacity under water deficit condition. *J. Phytol.* **2019**, *11*, 10–15.

52. Ofori, A.; Padi, F.K.; Akperter, A.; Adu-Gyamfi, P.; Dadzie, M.A.; Amoah, F.M. Variability of survival and yield traits in cacao (*Theobroma cacao* L.) clones under marginal field conditions in Ghana. *J. Crop Improv.* **2017**, *31*, 847–861. [\[CrossRef\]](#)
53. Lambers, H.; Chapin, F.S., III; Pons, T.L. *Plant Physiological Ecology*, 2nd ed.; Springer: New York, NY, USA, 1988; pp. 11–99.
54. Valladares, F.; Skillman, J.B.; Pearcy, R.W. Convergence in light capture efficiencies among tropical forest understory plants with contrasting crown architectures: A case of morphological compensation. *Am. J. Bot.* **2002**, *89*, 1275–1284. [\[CrossRef\]](#) [\[PubMed\]](#)
55. Valladares, F.; Niinemets, Ü. Shade tolerance, a key plant feature of complex nature and consequences. *Annu. Rev. Ecol. Evol. Syst.* **2008**, *39*, 237–257. [\[CrossRef\]](#)
56. Givnish, T.J. Adaptation to sun and shade: A whole-plant perspective. *Funct. Plant Biol.* **1988**, *15*, 63–92. [\[CrossRef\]](#)
57. Evans, J.; Poorter, H. Photosynthetic acclimation of plants to growth irradiance: The relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant Cell Environ.* **2001**, *24*, 755–767. [\[CrossRef\]](#)
58. Alvim, P.T. Cacao. In *Ecophysiology of Tropical Crops*, 1st ed.; Academic Press: London, UK, 1977; pp. 279–313.
59. Gutschick, V.P.; Wiegand, F.W. Optimizing the canopy photosynthetic rate by patterns of investment in specific leaf mass. *Am. Nat.* **1998**, *132*, 67–86. [\[CrossRef\]](#)
60. Lambers, H.; Poorter, H. Inherent variation in growth-rate between higher-plants—A search for physiological causes and ecological consequences. *Adv. Ecol. Res.* **1992**, *23*, 187–261.
61. Grime, J.P. *Plant Strategies, Vegetation Processes, and Ecosystem Properties*, 2nd ed.; John Wiley & Sons: Chichester, UK, 2006; p. 418.
62. Saavedra, F.; Jordan Peña, E.; Schneider, M.; Naoki, K. Effects of environmental variables and foliar traits on the transpiration rate of cocoa (*Theobroma cacao* L.) under different cultivation systems. *Agrosystem* **2020**, *94*, 2021–2031. [\[CrossRef\]](#)
63. Martins, S.C.; Galmes, J.; Cavatte, P.C.; Pereira, L.F.; Ventrella, M.C.; Damatta, F.M. Understanding the low photosynthetic rates of sun and shade coffee leaves: Bridging the gap on the relative roles of hydraulic, diffusive and biochemical constraints to photosynthesis. *PLoS ONE* **2014**, *9*, e95571. [\[CrossRef\]](#) [\[PubMed\]](#)
64. Amaral, J.A.T.; Rena, A.B.; Amaral, J.F.T. Crescimento vegetativo sazonal do cafeeiro e suas relações com fotoperíodo, frutificação, resistência estomática e fotossíntese. *Pesqui. Agropecu. Bras.* **2006**, *4*, 377–384. [\[CrossRef\]](#)
65. Wright, I.J.; Reich, P.B.; Westoby, M.; Ackerly, D.D.; Baruch, Z.; Bongers, F.; Cavender-Bares, J.; Chapin, T.; Cornelissen, J.H.C.; Villar, R.; et al. The worldwide leaf economics spectrum. *Nature* **2004**, *428*, 821–827. [\[CrossRef\]](#) [\[PubMed\]](#)
66. Lahive, F.; Handley, L.R.; Hadley, P.; Daymond, A.J. Climate change impacts on cacao: Genotypic variation in responses of mature cacao to elevated CO₂ and water deficit. *Agronomy* **2021**, *11*, 818. [\[CrossRef\]](#)
67. Carr, M.K.V.; Lockwood, G. The water relations and irrigation requirements of cocoa (*Theobroma cacao* L.): A review. *Exp. Agric.* **2011**, *47*, 653–676. [\[CrossRef\]](#)
68. Abo-Hamed, S.; Collin, H.A.; Hardwick, K. Biochemical and physiological aspects of leaf development in cocoa (*Theobroma cacao* L.) VII. Growth, orientation, surface structure and water loss from developing flush leaves. *New Phytol* **1983**, *95*, 9–17. [\[CrossRef\]](#)
69. Voltan, R.B.Q.; Fahl, J.I.; Carelli, M.L.C. Variação na anatomia foliar de cafeeiros submetidos a diferentes intensidades luminosas. *Rev. Bras. Fis. Veg.* **1992**, *4*, 99–105.
70. Bai, T.; Li, Z.; Song, C.; Song, S.; Jiao, J.; Liu, Y.; Dong, Z.; Zheng, X. Contrasting Drought Tolerance in Two Apple Cultivars Associated with Difference in Leaf Morphology and Anatomy. *Am. J. Plant Sci.* **2019**, *10*, 709–722. [\[CrossRef\]](#)
71. Ennajeh, M.; Vadel, A.M.; Cochard, H.; Khemira, H. Comparative impacts of water stress on the leaf anatomy of a drought-resistant and a drought-sensitive olive cultivar. *Hortic. Sci. Biotechnol.* **2010**, *85*, 289–294. [\[CrossRef\]](#)
72. Zakariyya, F.; Setiawan, B.; Susilo, A.W. Stomatal, proline, and leaf water status characters of some cocoa clones (*Theobroma cacao* L.) on prolonged dry season. *Pelita Perkeb.* **2016**, *33*, 109–117. [\[CrossRef\]](#)
73. Brodribb, T.J.; Feild, T.S.; Jordan, G.J. Leaf maximum photosynthetic rate and venation are linked by hydraulics. *Plant Physiol.* **2007**, *144*, 1890–1898. [\[CrossRef\]](#) [\[PubMed\]](#)
74. Brodribb, T.J.; Feild, T.S.; Sack, L. Viewing leaf structure and evolution from a hydraulic perspective. *Funct. Plant Biol.* **2010**, *37*, 488–498. [\[CrossRef\]](#)
75. Zanetti, L.V.; Milanez, C.R.D.; Gama, V.N.; Aguilar, M.A.G.; Souza, C.A.S.; Campostrini, E.; Ferraz, T.M.; Figueiredo, F.A.M.M.d.A. Leaf application of silicon in young cacao plants subjected to water deficit. *Pesqui. Agropecu. Bras.* **2016**, *51*, 215–223. [\[CrossRef\]](#)
76. Brooks, E.R.; Guard, A.T. Vegetative anatomy of *Theobroma cacao*. *Bot. Gaz.* **1952**, *113*, 444–454. [\[CrossRef\]](#)
77. Hanba, Y.T.; Miyazawa, S.I.; Terashima, I. The influence of leaf thickness on the CO₂ transfer conductance and leaf stable carbon isotope ratio for some evergreen tree species in Japanese warm-temperate forests. *Funct. Ecol.* **1999**, *13*, 632–639. [\[CrossRef\]](#)
78. Jones, H.G. *Plants and Microclimate: A Quantitative Approach to Environmental Plant Physiology*, 3rd ed.; Cambridge Univ. Press: Cambridge, UK, 2013; pp. 122–152.
79. Jordaan, A.; Kruger, H. Structure of xerophytic plants from Southern Africa leaf anatomy of *Antizoma miersiana* and *Diospyros ramulosa*. *Isr. J. Bot.* **1992**, *41*, 57–65.
80. Chapotin, S.M.; Holbrook, N.M.; Morse, S.R.; Gutiérrez, M.V. Water relations of tropical dry forest flowers: Pathways for water entry and the role of extracellular polysaccharides. *Plant Cell Environ.* **2009**, *26*, 623–630.
81. Clifford, S.C.; Arndt, S.K.; Popp, M.; Jones, H.G. Mucilages and polysaccharides in Ziziphus species (Rhamnaceae): Localization, composition and physiological roles during drought-stress. *J. Exp. Bot.* **2002**, *53*, 131–138. [\[CrossRef\]](#) [\[PubMed\]](#)
82. Nakayama, L.H.I.; Soares, M.K.M.; Appezzato-Da-Glória, B. Contribuição ao estudo anatômico da folha e do caule do cacaueiro (*Theobroma cacao* L.). *Sci. Agric.* **1996**, *53*, 73. [\[CrossRef\]](#)
83. Shahidi, F.; Naczk, M. *Phenolics in Foods and Nutraceuticals*, 2nd ed.; CRC Press: Boca Raton, FL, USA, 2003; p. 576.

84. Vizzotto, M.; Krolow, A.C.R.; Weber, G.E.B. Metabólitos secundários encontrados em plantas e sua importância. *Embrapa Clima Temperado Pelotas Braz.* **2010**, *316*, 1–17.
85. Shatil-Cohen, A.; Attia, Z.; Moshelion, M. Bundle-sheath cell regulation of xylem-mesophyll water transport via aquaporins under drought stress: A target of xylem-borne ABA? *Plant J.* **2011**, *67*, 72–80. [[CrossRef](#)] [[PubMed](#)]
86. Lynch, D.J.; Mcinerney, F.A.; Kouwenberg, L.L.; Gonzalez-Meler, M.A. Plasticity in bundle sheath extensions of heterobaric leaves. *Am. J. Bot.* **2012**, *99*, 1197–1206. [[CrossRef](#)] [[PubMed](#)]
87. Hsiao, T.C. Plant responses to water stress. *Ann. Rev. Plant Physiol.* **1973**, *24*, 519–570. [[CrossRef](#)]
88. Ahnert, D.; Melo, H.L.; Santos, F.F.J.; Lima, L.R.; Baligar, V.C. Melhoramento genético e produtividade do cacauzeiro no Brasil. In *Cacau, Cultivo, Pesquisa e Inovação*, 1st ed.; Souza Júnior, J.O., Ed.; Editus: Ilhéus, BA, Brazil, 2018; pp. 151–182.
89. Alonso, R.S. Caracterização Fisiológica, Morfoanatômica e Histoquímica dos Órgãos Vegetativos de Clones de Cacauzeiro. Master's Thesis, Universidade Estadual de Santa Cruz, Ilhéus, BA, Brazil, 26 February 2021.

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