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How Do Different Cocoa Genotypes Deal with Increased Radiation? An Analysis of Water Relation, Diffusive and Biochemical Components at the Leaf Level

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Abstract: The cultivation of cocoa (*Theobroma cacao* L.) is traditionally managed under shade because of its photosynthetic characteristics; however, its behavior can vary according to the genotype and environmental conditions where it is grown. In this sense, here, we explore the possible mechanisms of protection against radiation stress and how these mechanisms are affected by variation between cocoa genotypes. Therefore, we evaluate the effect of the radiation level (H_{PAR}, 2100 ± 46 mol m^{-2} s^{-1}; M_{PAR}, 1150 ± 42 mol m^{-2} s^{-1}; L_{PAR}, 636 ± 40 mol m^{-2} s^{-1}) on the water status and gas exchange in plants of different cocoa genotypes (CCN-51, ICS-1, ICS-95, LUKER-40 and LUKER-50), and the occurrence of photoinhibition of PSII (as a marker of photodamage), followed by a characterization of the protection mechanisms, including the dynamics of photosynthetic pigments and enzymatic and non-enzymatic antioxidant systems. We found significant changes in the specific leaf area (*SLA*) and the water potential of the leaf (Ψ_L) due to the level of radiation, affecting the maximum quantum yield of PSII (F_v/F_m), which generated dynamic photoinhibition processes (PIDyn). Cocoa genotypes showed the lowest Light-saturated maximum net carbon assimilation rate (A_{max}) in H_{PAR}. Moreover, the maximum carboxylation rate (V_{cmax}) was negatively affected in H_{PAR} for most cocoa genotypes, indicating less RuBisCO activity except for the ICS-95 genotype. The ICS-95 showed the highest values of V_{cmax} and maximum rate of regeneration of ribulose-1,5-bisphosphate (RuBP) controlled by electron transport (J_{max}) under H_{PAR}. Hence, our results show that some genotypes were acclimated to full sun conditions, which translated into greater carbon use efficiency due to the maximization of photosynthetic rates accompanied by energy dissipation mechanisms.

Keywords: *Theobroma cacao*; irradiance; gas exchange; photoinhibition; chlorophyll fluorescence

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1. Introduction

The level of solar radiation is a factor that can limit some physiological processes on crops, such as photosynthesis [1,2]. Crops under shade conditions adjust a suite of anatomical, physiological and biochemical traits to increase the efficiency of carbon fixation [3]. Among these traits are found the specific leaf area (SLA), the chlorophyll a/b ratio (chloroplast level) and the rate of light-saturated net photosynthesis (A_{max}) [4–6]. An increased radiation level above the light saturation point can cause damage to the photosynthetic apparatus [7–9], even causing dynamic or chronic photoinhibition when the plant is not able to dissipate the excess energy adequately [10–12], which affects crop production. The excess energy could cause damage to the photosynthetic reaction centers causing chronic photoinhibition, considered a slower reversible loss of reaction center function that depends on the repair and recovery rates of the protein [12,13]. If the exposure

process is more extensive, high irradiation can lead to irreversible oxidation of chlorophyll (Chl) and a loss of chloroplast function due to the formation of reactive oxygen species (ROS) [14]. Faced with this stressful situation, the plant makes biochemical adjustments related to the content of photosynthetic pigments (a, b and carotenoids), protein content, antioxidant system, sugar content and malondialdehyde (MDA) [8,15–17].

Cocoa (*Theobroma cacao* L.) is native to the rainforests of the Amazon [18]. It is considered one of the world's most important perennial crops, with the largest production coming from West Africa, followed by South East Asia and Latin America [19]. It is a species that adapts to shade conditions that result in photosynthetic characteristics related to a low point of saturation and light compensation [20–28]. For this reason, cocoa crops in most cocoa-producing regions of the world tend to be established in shaded environments. However, in recent years, different physiological behaviors have been observed when cocoa is grown in full sun, specifically in areas where most of the year there is cloud cover and low demand for air evaporation [6,29], characteristic management in regions of Indonesia [30] and Malaysia [31] in the Ecuadorian Amazon [32] and on the Atlantic coast of Brazil [8].

In the Amazon, unlike other cocoa-producing areas in Colombia (Santander, Arauca, and Huila), the annual average radiation is only 3–4 sunshine hours per day [33] due to its high cloud cover. This situation can cause, in addition to the type of agroforestry system [34], variations in microclimatic conditions [35]. Due to these prevailing environmental conditions, there is some uncertainty about the real need to establish cocoa plantations under shade conditions. In that sense, to maximize cocoa production [23], it is necessary to carry out studies on the physiological behavior of different genotypes of cocoa under different environmental and management conditions, especially when there is inconsistency in the optimal shading conditions that allow for increased growth [36]. In this regard, some studies suggest genotypic differences in relation to radiation variation [36–38]. Accordingly, our main objective was to explore the potential mechanisms of protection against radiation stress and how these mechanisms are affected by variation among cocoa genotypes. To achieve these objectives, we first evaluated the effect of the radiation level (H_{PAR}, 2100 ± 46 mol m^{-2} s^{-1}; M_{PAR}, 1150 ± 42 mol m^{-2} s^{-1}; L_{PAR}, 636 ± 40 mol m^{-2} s^{-1}) on the water status and gas exchange in plants of different cocoa genotypes (CCN-51, ICS-1, ICS-95, LUKER-40 and LUKER-50). We also evaluated the occurrence of photoinhibition of PSII (as a marker of photodamage), followed by a characterization of the protection mechanisms, including the dynamics of photosynthetic pigments, enzymatic and non-enzymatic antioxidant systems.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Measurements were taken for cacao plants under different radiation levels in the Centro de Investigaciones Amazónicas CIMAZ Macagual-Universidad de la Amazonia (137′ N and 7536′ W at 360 m a.s.l.), Colombia. The climate is warm–humid, typical of the ecosystem of tropical rainforests, with a mean annual temperature of 25.5 °C, precipitation of 3800 mm, relative humidity of 84% and 1200 sunshine hours $year^{-1}$. Five cocoa genotypes (CCN-51, ICS-1, ICS-95, LUKER-40 and LUKER-50) were used, which were grafted onto plants of the genotype IMC67, which had previously germinated in a nursery. At the age of 7 months (3 months of growth of IMC 67 + 4 months in grafting), the cocoa seedlings were transplanted in plastic bags with a capacity of 100 L with a homogeneous mixture of soil (3:1:1 mixture of clay-rich soil, sand and organic substrate) under a radiation level of 600 mol m^{-2} s^{-1}. Ten plants of each genotype were subjected to a period of 180 days of acclimatization at each of the radiation levels with constant water availability close to field capacity ($\Psi_L \sim -0.2 \pm 0.01$ MPa using soil water potential sensor MPS-2 Decagon Devices, Inc., Pullman, WA, USA). The three levels of radiation considered were high mean daily incident photosynthetically active radiation (H_{PAR}, 2100 ± 46 mol m^{-2} s^{-1}) that was in full sun, medium mean daily incident photosynthetically active radiation (M_{PAR}, 1150 ± 42 mol m^{-2} s^{-1}), and low mean daily incident photosynthetically active radiation

(L_{PAR}, 636 ± 40 mol m^{-2} s^{-1}) values that were obtained from the measurement during the adaptation period of the plants between 11:00 am and 1:00 pm using the AccuPAR ceptometer LP-80 (Decagon Devices Inc., Pullman, WA, USA). To simulate the radiation levels in M_{PAR} and L_{PAR}, we used poly shade meshes branded "Colmallas®". A randomized complete block (RCB) design with factorial arrangement (3 levels of radiation × 5 genotypes) was used for the study. The plot was composed of ten plants by cocoa genotypes under each level of radiation.

2.2. Leaf Water Potential (Ψ_L) and Specific Leaf Area

The assessments of Ψ_L were performed employing a PMS Model 1000 pressure chamber (PMS Instrument Company, Corvallis, OR, USA) in the second or third mature leaf from the apex of the orthotropic axis between 04:00 and 05:00 h (solar time) in three plants for each cocoa genotype under different levels of radiation. Specific leaf area (SLA) was determined using six leaf discs (3.14 cm^2), omitting the mid-vein for each leaf that had been previously employed to measure gas exchange and Chl_a fluorescence (n = 810 corresponding to 3 plants per genotype (5 genotypes) per treatment (3 PAR levels), 3 leaves per plant and 18 discs per plant). The discs were dried at 70 °C until that a constant mass was achieved, and the SLA determined as the ratio between the leaf disc area and its respective dry mass [39].

2.3. Photosynthetic Light- and CO_2-Response Curves of Different Genotypes of Cacao Grown under Varying Levels of Radiation

For measuring the gas exchange, leaves attached to the plant corresponding to the second or third mature leaf from the apex were assessed using an infrared gas analyzer CIRAS-3 Portable Photosynthesis System (PP Systems Inc. Amesbury, MA, USA) was used as described before by Suárez et al. [6,40]. The leaf cuvette environmental conditions were set with a vapor pressure deficit (VPD) ranging from 1.0 to 1.5 kPa and a constant temperature of 25 °C. Measurements were performed between 08:00 and 11:00 h (solar time), at a partial concentration of CO_2 of 400 ppm, and under photosynthetically active radiation (PAR) provided by the LED light source of the cuvette. The stabilization time for gas exchange data collection was 10 min. The photosynthetic (A) response curves to PAR intensity (hereafter, A/PAR) were obtained by increasing PAR in 10 steps from 0 to 2000 µmol m^{-2} s^{-1}. The stabilization time at each point of the A/PAR was 3 min with CO_2 stability of 400 ± 0.1 ppm, a process that was carried out similarly for each of the cocoa genotypes grown at each level of radiation. Initially, in the chamber, leaves were exposed to a VPD between 1.0 and 1.5 kPa, a leaf temperature of 25 °C, and a partial concentration of CO_2 of 50 ppm for 5 min, allowing the opening of the stomata; successively, A/PAR curves were generated at a partial concentration of CO_2 of 400 ppm. Photosynthetic limitations in each genotype, resulting from microclimatic conditions of radiation level, were stablished using the above data to estimate different parameters derived from the A/PAR curves, light compensation point (LCP), light-saturated maximum net carbon assimilation rate (A_{max}), light saturation point (LSP), dark respiration rates (R_d) and apparent quantum efficiency (Φ_{PAR}) that was calculated from the slope of the initial linear portion of the A/PAR curve [41] recommended.

Photosynthetic assimilation response curves to internal CO_2 concentration (hereafter, A/C_i) were obtained at a PAR of 500 µmol m^{-2} s^{-1} (based on the A/PAR curves), at 25 °C and ambient O_2 concentration as recommended byLong and Bernacchi [42] on leaves attached to the plant located on the second or third mature leaf from the apex. The stabilization time for gas exchange data collection was 10 min. Measurements began a partial concentration of CO_2 of 400 ppm, which was gradually diminished until 50 ppm and then increased in 15 steps until 1600 ppm of partial concentration of CO_2 [43]. Following Flexas et al.'s [44] recommendations, leakage errors were corrected by measuring the CO_2 response curves in dead leaves. The Maximum rate of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) carboxylation (V_{cmax}), the maximum rate of electron

transport driving the regeneration of ribulose-1,5-bisphosphate (RuBP) (J_{max}), and leaf respiration under light conditions (R_D) were estimated from each A/C_i curve.

2.4. Chlorophyll (Chl_a) Parameters of Different Genotypes of Cacao Grown under Varying Levels of Radiation

Measurements were done on the same leaves employed to measure gas exchange differences using the Chlorophyll Fluorescence Module (CFM-3) adapted for the infrared gas analyzer CIRAS-3 (PP Systems Inc., Amesbury, MA, USA) was used. The CFM-3 provides chlorophyll fluorescence measurements using the pulse-amplitude modulation (PAM) technique. The maximum quantum yield of PSII (F_v/F_m) was established in leaves under complete darkness at predawn (04:00 h solar time), and midday (13:00 h solar time, the dark adaptation time was 45 min) by exposing the leaves to a saturating pulse of light (6000 µmol m^{-2} s^{-1}; 1 s). The leaf tissues were exposed to actinic photon irradiance (110 µmol m^{-2} s^{-1}) for 120 s to obtain the steady-state fluorescence yield (F_s), after which a saturating white light pulse (2400 µmol µmol m^{-2} s^{-1}; 0.8 s) was applied to achieve the light-adapted maximum fluorescence (F'_m).

The actual PSII quantum efficiency (Φ_{PSII}) that assesses the proportion of light absorbed by the PSII light-harvesting antenna used in the photochemical process [45], was calculated in the light-adapted state as follows:

$$\Phi_{PSII} = (F_m' - F_s)/F_m' \quad (1)$$

where F_s represents the measured fluorescence immediately before the application of light pulses.

The apparent electron transport rate (ETR) that indicates the overall photosynthetic capacity in vivo, was calculated as follows [46]:

$$ETR = PAR \times 0.84 \times 0.5 \times \Phi_{PSII} \quad (2)$$

where the fraction of Φ_{PSII} centers, are in the open state according to the Lake model of the PSII photosynthetic unit [46] and qP is photochemical quenching coefficient:

$$qP = (F_m' - F_T)/(F_m' - F'_0) \quad (3)$$

where F_T is the steady-state yield of fluorescence in the light; the F_0' parameter is assessed after an introduction of far-red illumination on light-adapted leaves when all of the PSII reaction centers and acceptors of electrons are oxidized once again, employing a far-red light lighting [46]. Furthermore, the non-photochemical quenching of Chl_a fluorescence (NPQ), which indicates heat dissipation of Chl excitation energy through the PSII light-harvesting antenna, was also estimated [46]:

$$NPQ = (F_m - F_m')/F_m' \quad (4)$$

where F_m is maximal fluorescence in the dark.

The photoinhibition indices of the PSII were also estimated according to Werner et al. [11], including (A) chronic photoinhibition (PIChr), representing the percent reduction in F_v/F_m at each radiation level for each genotype relative to the maximal F_v/F_m obtained during the entire experiment; (B) dynamic photoinhibition (PIDyn), representing the decline in F_v/F_m that is fully reversible overnight, being calculated as the percent reduction in midday $F_{/v}/F_{/m}$ relative to F_v/F_m at each t radiation level for each genotype, relative to the maximal F_v/F_m from the entire experiment; (C) was also estimated total photoinhibition:

$$PIChr = [(F_v/F_m)\ max - (F_v/F_m)\ pd]/(F_v/F_m)max \times 100\% \quad (5)$$

$$PIDyn = [(F_v/F_m)\ pd - (F_v/F_m)\ mid]/(F_v/F_m)max \times 100\% \quad (6)$$

$$PITotal = PIChr + PIDyn \quad (7)$$

2.5. Biochemical Assays

In all pigment and enzymatic analyses, the leaf discs were collected between 12:00 and 13:00 h (solar time) when photosynthetic rates were maximal [47]. We followed the protocol described by Lichtenthaler [48] to estimate the levels of total Chl (Chl_t), Chl_a and Chl_b, and carotenoids for each leaf using six leaf discs (3.14 cm^2) from the same leaves that had been used previously to calculate SLA. Between one of the key enzymes, glutathione reductase (GR; EC 1.6.4.2) was assayed as described in Pinheiro et al. [49]. Cellular damages were analyzed through electrolyte leakage (assayed immediately after leaf sampling) when plants reached leaf Ψ_L of −1.5 and −3.0 MPa and malondialdehyde (MDA) accumulation (which expresses lipid peroxidation), as reported earlier [50].

2.6. Data Analysis

The Michaelis-Menten hyperbolic constant was used to adjust the A/PAR curves; the parameters A_{max}, LSP, LCP, R_d, and Φ_{PAR} were carried out during the day and estimated following the equations described by Lobo et al. [51]. Evaluation of the A/C_i curve and stimation of the V_{cmax}, J_{max}, and R_D was performed emplying the model created by Farquhar et al. [52] using the plantecophys package in R [53]. A generalized linear model (GLM) was adjusted for the different parameters derived from the A/PAR and A/C_i curves for each genotype at each radiation level and the interaction of these (fixed factor). The plant and leaf were included as random factors (n = 16). Similarly, a GLM was made for SLA, photosynthetic pigments, including the level of radiation as the fixed factor. The plant, leaf and leaf discs were included as random factors. For the variables (leaf water potential and chlorophyll fluorescence) that were measured both at pre-warming and midday, a GLM model was performed, where the radiation level, genotype, time during the day and the interaction of these were analyzed as fixed factors. The plant and leaf were included as random factors. The assumptions of normality and homogeneity of variance were evaluated using an exploratory residual analysis. Differences between mean values of each genotype cacao plant responses under the level of radiation (fixed factor) were analyzed with the LSD Fisher's post hoc test at a significance of α = 0.05. Analyses of GLM were performed using the lme function in the nlme package [54] in R language software, version 4.0.0 [55], and using the interface in InfoStat [56].

3. Results

3.1. Leaf Water Potential and Specific Leaf Area

Independent of the cocoa genotypes and the radiation level studied, the Ψ_L before dawn (-0.08 ± 0.01 MPa $p > 0.05$) was significantly lower than that found at midday (-1.06 ± 0.07 MPa $p < 0.05$). Additionally, an increase in the water deficit was found by increasing the PAR level at midday. When comparing cocoa genotypes, LUK-40 was the most sensitive in its water status, contrary to the behavior presented for ICS-95, which presented the lowest Ψ_L values under the different levels of radiation at midday (Figure 1). In general, SLA tended to increase with decreasing radiation and the change was significant except for ICS-95 and LUK-50 (Figure 2).

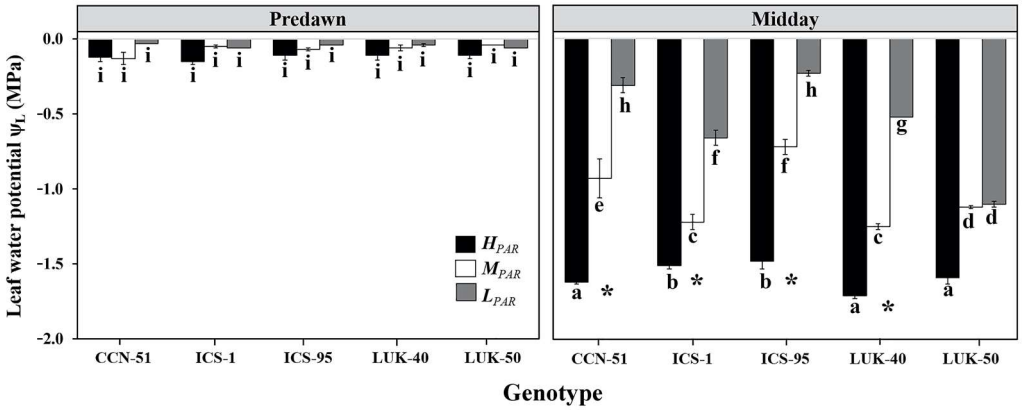

Figure 1. The leaf water potential (Ψ_L; MPa) at predawn and midday for five genotypes of cacao in three levels of radiation considered were high mean daily incident photosynthetically active radiation (H_{PAR}, 2100 ± 46 mol m^{-2} s^{-1}) that was in full sun, medium mean daily incident photosynthetically active radiation (M_{PAR}, 1150 ± 42 mol m^{-2} s^{-1}) and low mean daily incident photosynthetically active radiation (L_{PAR}, 636 ± 40 mol m^{-2} s^{-1}). [a,b,c]: Values in bars with different letters indicate significant differences between genotypes of cacao × radiation level (post hoc LSD Fisher, $p < 0.05$). *: Means statistical differences between radiation levels in each cocoa genotype. The results include means ± SE (n = 4).

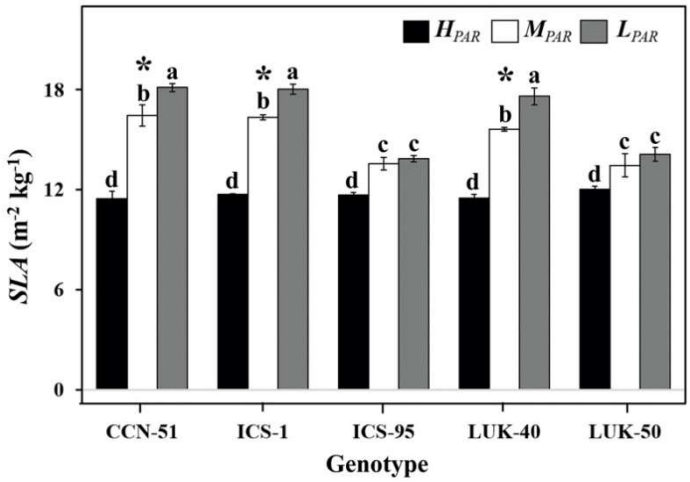

Figure 2. The specific leaf area (SLA; m^2 kg^{-1}) for five genotypes of cacao in three levels of radiation considered were high mean daily incident photosynthetically active radiation (H_{PAR}, 2100 ± 46 mol m^{-2} s^{-1}) that was in full sun, medium mean daily incident photosynthetically active radiation (M_{PAR}, 1150 ± 42 mol m^{-2} s^{-1}) and low mean daily incident photosynthetically active radiation (L_{PAR}, 636 ± 40 mol m^{-2} s^{-1}). [a,b,c]: Values in bars with different letters indicate significant differences between genotypes of cacao × radiation level (post hoc LSD Fisher, $p < 0.05$). *: Means statistical differences between radiation levels in each cocoa genotype. The results include means ± SE (n = 54 leaf discs). Statistics are defined as in Figure 1.

3.2. Photosynthetic Light- and CO_2-Response Curves of Different Genotypes of Cacao Grown under Varying Levels of Radiation

Significant differences ($p < 0.05$) were observed in parameters obtained from the light and CO_2 response curves in the interaction between genotypes of cacao and radiation level. The increase in radiation level had a significant effect on different photosynthetic characteristics (Figure 3). Cocoa genotypes such as ICS-95 and LUK-40 presented a greater response (both on the area and mass bases) in the lower radiation levels (Figure 3a,b). For $R_{d,}$ no specific trend was found between the radiation levels, being lowest for LUK-40 in L_{PAR} (Figure 3c). The highest LSP values were mainly obtained in the plants that underwent L_{PAR}; however, LUK-40 presented a contrary behavior and ICS-95 exhibited similar means

in H_{PAR} and L_{PAR} (Figure 3d). For the LCP, a consistent trend was not clear, and only ICS-95 and LUK-50 showed higher values in H_{PAR} and M_{PAR}, respectively. The greatest efficiency in terms of electron transfer (Φ_{PAR} Figure 3f) (revealed as the highest maximum rate of regeneration of ribulose-1.5-bisphosphate controlled by electron transport) was observed with three genotypes (LUK-50, ICS-1 and CCN-51) in H_{PAR}, while LUK-40 exhibited opposite behavior. V_{cmax} did not respond to PAR, while J_{max} was significantly higher for ICS-1 and ICS-95 by increasing PAR. Finally, R_D was found lower in H_{PAR} for all genotypes, except ICS1 that showed the lowest values in L_{PAR}.

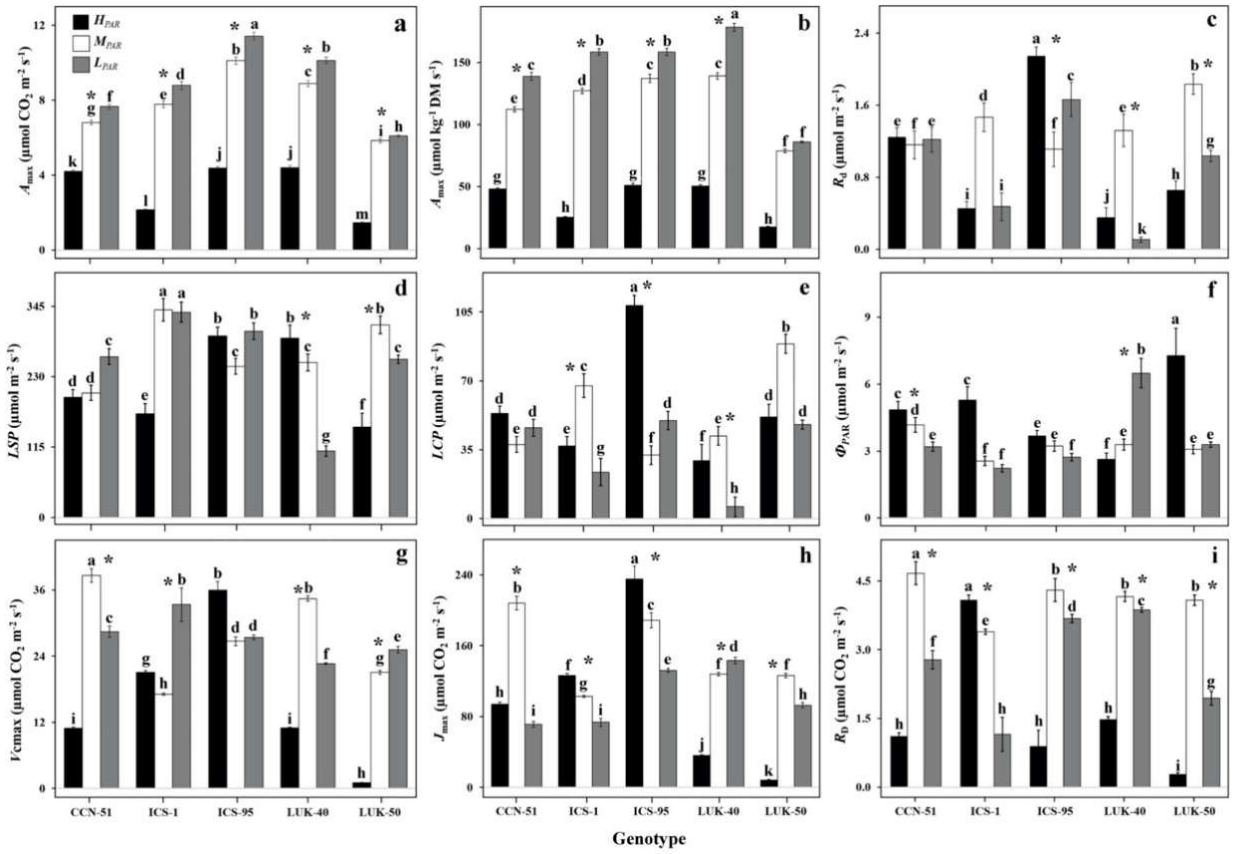

Figure 3. Parameters derived from photosynthetic light (A/PAR) and CO_2 (A/C_i) response curves for five genotypes of cacao in three levels of radiation considered were high mean daily incident photosynthetically active radiation (H_{PAR}, 2100 ± 46 mol m^{-2} s^{-1}) that was in full sun, medium mean daily incident photosynthetically active radiation (M_{PAR}, 1150 ± 42 mol m^{-2} s^{-1}) and low mean daily incident photosynthetically active radiation (L_{PAR}, 636 ± 40 mol m^{-2} s^{-1}). (**a**,**b**). A_{max}: Light-saturated maximum net carbon assimilation rate; DM: Dry mass; (**c**). R_d: Dark respiration rate; (**d**). LSP: Light saturation point; (**e**). LCP: Light compensation point; (**f**). Φ_{PAR}: Quantum efficiency; (**g**). V_{cmax}: Maximum carboxylation rate; (**h**). J_{max}: Maximum rate of regeneration of ribulose-1,5-bisphosphate (RuBP) controlled by electron transport; (**i**). R_D: Leaf respiration in light conditions. ^{a,b,c}: Values in bars with different letters indicate significant differences between genotypes of cacao × radiation level (post hoc LSD Fisher, $p < 0.05$). *: Means statistical differences between radiation levels in each cocoa genotype. The results include means ± SE ($n = 4$). Statistics are defined as in Figure 1.

3.3. Chlorophyll (Chl_a) Parameters of Different Genotypes of Cacao Grown under Varying Levels of Radiation

The mean values of F_v/F_m in predawn (0.80 ± 0.01) were statistically similar for most cocoa genotypes at the different radiation levels, except CCN-51 and ICS-95 in L_{PAR} (Figure 4a). However, at midday, the F_v/F_m values decreased to the extent that controlled dissipation mechanisms were generated as the dynamic PSII photoinhibition (PIDyn) gradually increased with increasing radiation intensity (Figure 4b). The increase in PAR

impacted photoinhibition in the different cocoa genotypes, being in most cases PIDyn (Figure 4b). Only at the L_{PAR} level for the ICS-95 genotype was it higher PIChr than PIDyn.

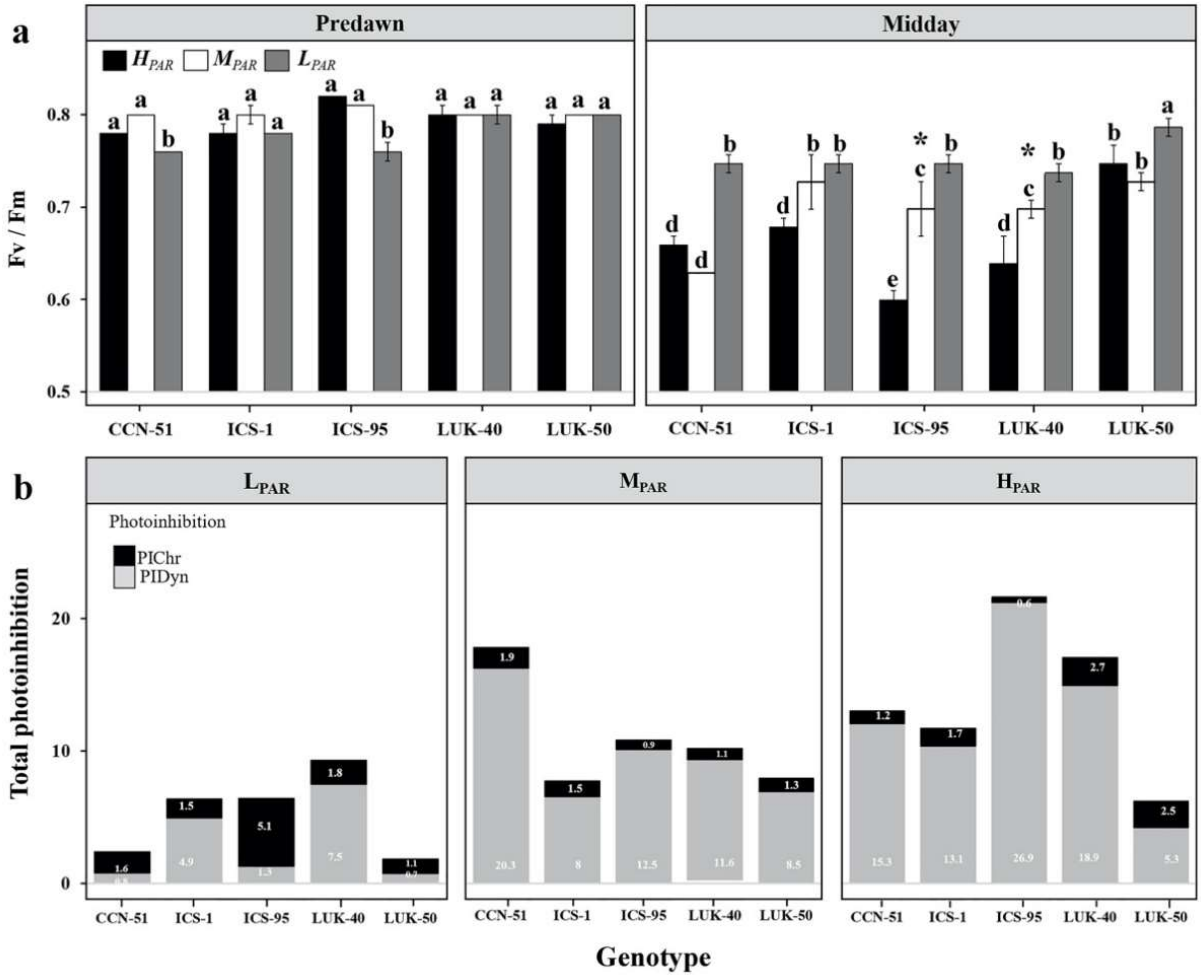

Figure 4. Parameters of chlorophyll a fluorescence analysis in five cocoa genotypes under different levels of radiation. (**a**). maximum quantum yield of PSII (F_v/F_m), (**b**). photoinhibition indices of the PSII. Chronic PSII photoinhibition (PIChr). Dynamic PSII photoinhibition (PIDyn). [a,b,c]: Values in bars with different letters indicate significant differences between genotypes of cacao × radiation level (post hoc LSD Fisher, $p < 0.05$). *: Means the statistical differences between the radiation levels in each of the cocoa genotypes at the different evaluation times. The results include means ± SE ($n = 4$). Statistics are defined as in Figure 1.

The electron transport rate (ETR) was higher in the plants of the cocoa genotypes grown in the L_{PAR}, with ICS-95 and LUK-50 showing the highest values (Figure 5). However, under H_{PAR} the Φ_{PSII} was reduced in the cocoa genotypes ICS-1, LUK-50 and CCN-51 (Figure 5). The photochemical quenching coefficient (qP) reduction was reduced by increasing PAR availability (x-axis) but was more pronounced at H_{PAR}, with ICS-95 and LUK-40 being the cocoa genotypes that performed best (Figure 5). Finally, among different genotypes, LUK-40 was found to dissipate the highest amount of energy in the form of heat (NPQ) in the different PAR levels (Figure 5).

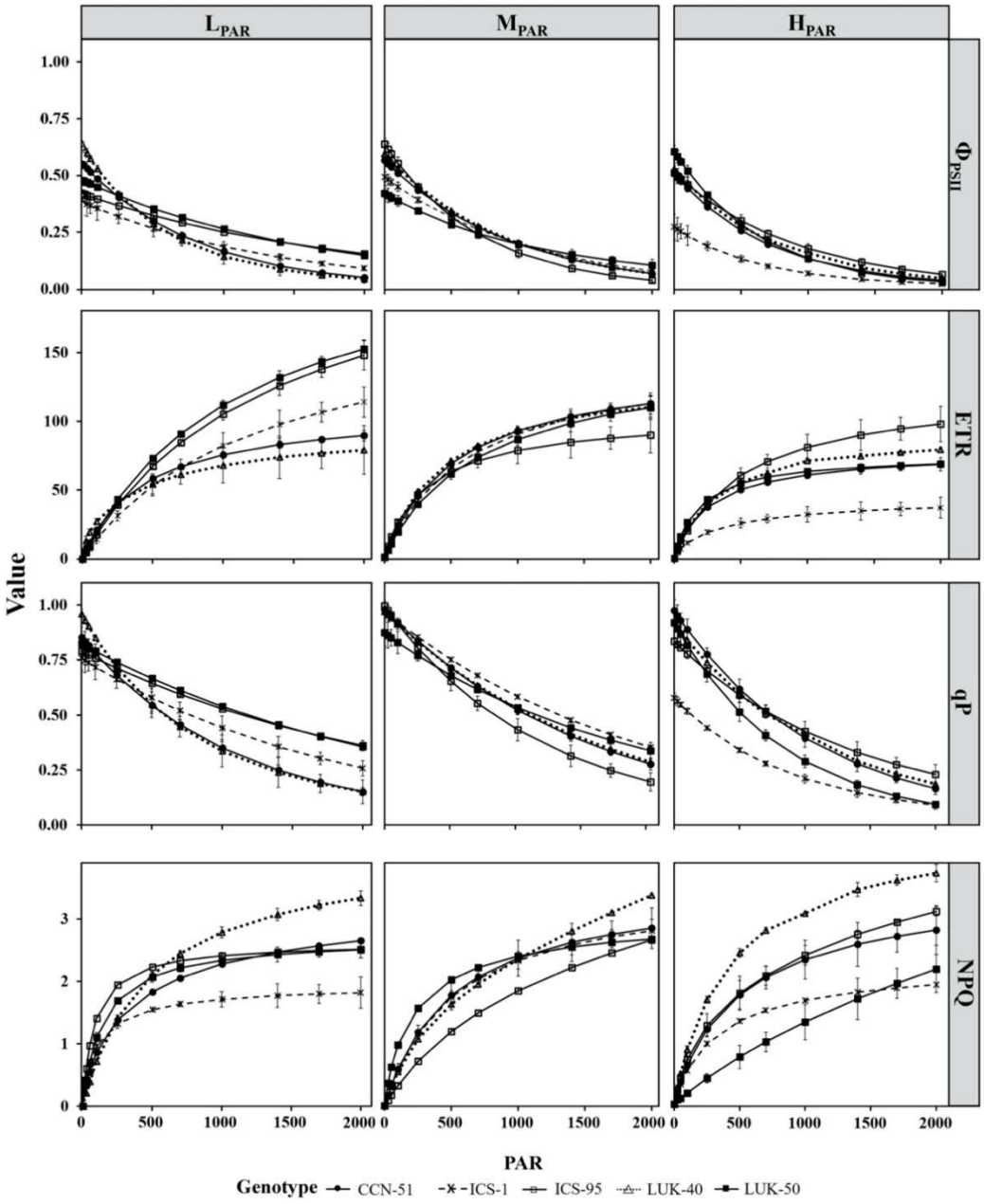

Figure 5. Chl fluorescence parameters in relation to PAR levels in cacao leaves for five genotypes of cacao in three radiation levels. Actual PSII quantum yield (ΦPSII); electron transport rate (ETR); photochemical quenching coefficient (qP); non-photochemical quenching coefficient (NPQ). The results include means ± SE (n = 4). Statistics are defined as in Figure 1.

It was found that the total concentration of Chl_t (a + b) was higher in the shaded leaves than in the sunlight ones, being statistically different between cocoa genotypes under different PAR levels (Figure 6). Because total *Chl* concentration decreased more than carotenoids, these parameters were lower in H_{PAR}. *GR* did not exhibit a clear trend, although CCN-51 and LUK-40 showed higher values. Electrolyte leakage and *MDA* showed similar behavior, with lower values in L_{PAR} (Figure 6).

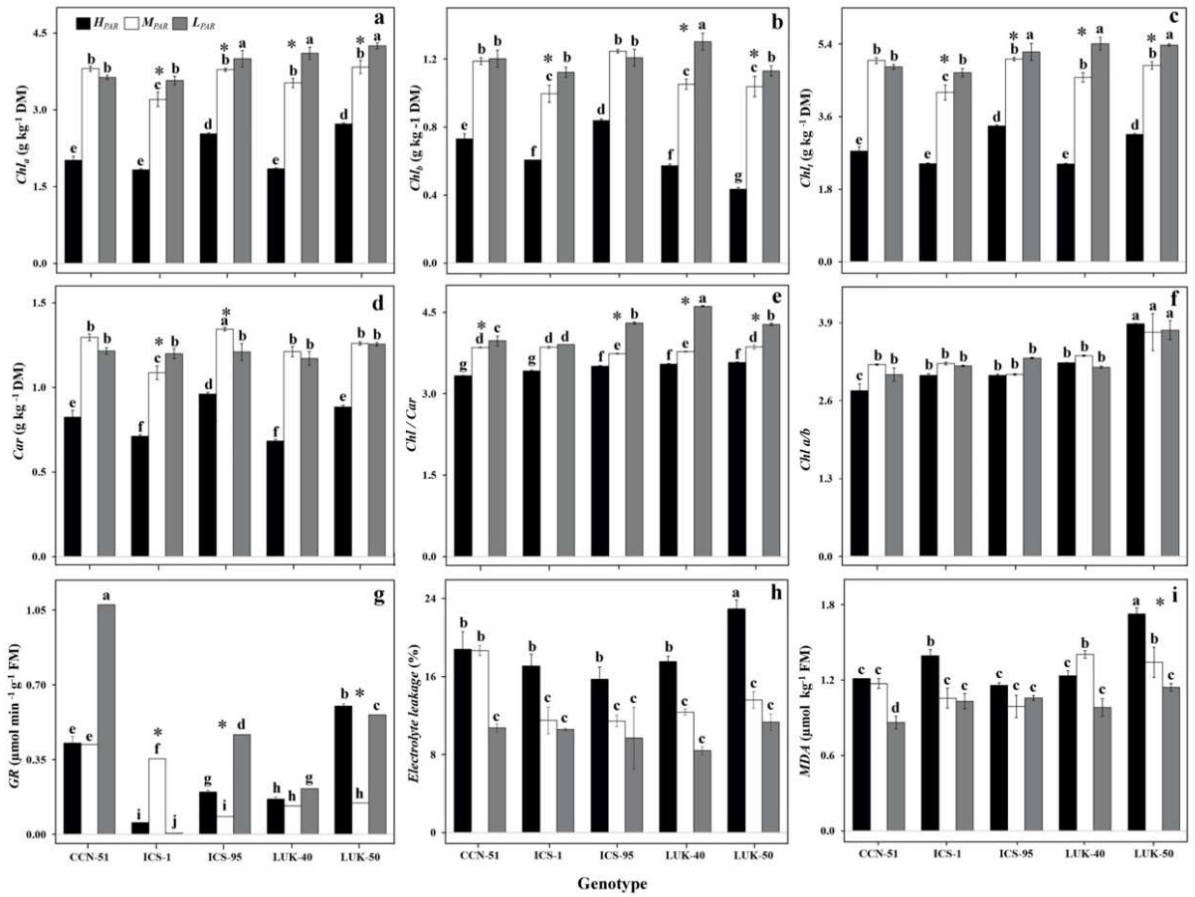

Figure 6. Biochemical assays for five genotypes of cacao in three levels of radiation. (**a**). Chl_a: chlorophyll a; (**b**). Chl_b: chlorophyll b; (**c**). Chl_t: Total chlorophyll; (**d**). Car: Carotenoid; (**e**). Chl_t/Car: Total chlorophyll/ carotenoids ratio; (**f**). Chl_a/Chl_b: chlorophyll a/b ratio; (**g**). glutathione reductase (GR; μmol min^{-1} g^{-1} FM); (**h**). electrolyte leakage (μmol kg^{-1} FM) and (**i**) concentration of malondialdehyde (MDA; μmol kg^{-1} FM). ^{a,b,c}: Values in bars with different letters indicate significant differences between genotypes of cacao × radiation level (post hoc LSD Fisher, $p < 0.05$). *: Means statistical differences between radiation levels in each cocoa genotype. The results include means ± SE ($n = 4$).

4. Discussion

We found good physiological performance at a low level of solar radiation for some cocoa genotypes as well as other genotypes exhibiting adequate acclimatization to relatively high solar radiation conditions. Plants in H_{PAR} that were subjected to harsh environmental conditions (high accumulated temperature, air–leaf *VPD*, and radiation loads) were affected by Ψ_L at midday, directly affecting carbon assimilation (A), contrary to what was found in L_{PAR}. When radiation levels increase, changes in environmental conditions are generated, mainly related to the increase in temperature, which has an impact on the water status of the plant [24,57], since reaching values below −1.5 MPa can have significant effects on photosynthesis [24,58]. Therefore, as a mechanism of adjustment to water deficit, cocoa plants reduce their stomatal conductance and thus carbon fixation [25]. Specifically, for all cocoa genotypes, a low level of A_{max} in H_{PAR} was found, and for most of them, V_{cmax} was also affected in H_{PAR}, suggesting less RuBisCO activity. However, the ICS-95 genotype showed the highest values of V_{cmax} and J_{max} under H_{PAR}.

In order to achieve adequate carbon fixation in L_{PAR} as well as H_{PAR}, adjustments were required in the capture, use and dissipation of light to provide photoprotection for the photosynthetic apparatus, thus avoiding the appearance of photoinhibitory processes. Under shaded conditions, electron transport rate (ETR) is reduced in plants; however, cocoa plants as a mechanism to increase light harvesting increase pigment content [4,6],

likewise, as a strategy to cope with the low PAR, a situation presented in plants under L_{PAR}. It has been reported that SLA is a trait related to the level of incident light [26] and specifically in cocoa cultivation when increasing the level of radiation the SLA is reduced [59,60], a characteristic presented in crops under monoculture compared to that found under agroforestry systems [61,62]. This SLA variation is a trait that may be related to the reduction in leaf thickness when plants are subjected to low light intensity. It can be interpreted as a strategy to obtain a larger surface area towards more efficient absorption and optimization of photon capture [7] and a way to reduce the transpiration rate [61].

Second, from the balance of energy use and/or dissipation capabilities, analyzed by the dynamic photoinhibition (PIDyn) and chronic (PIChr) estimates of the PSII, we found that mainly PIDyn increased when PAR was increased. The leaves of the cocoa genotypes in L_{PAR} showed similar capacities to those of the leaves in H_{PAR} against photoinhibition, as indicated by the strong upward regulation of *NPQ* when exposed to light, as well as the slight decrease in the F_v/F_m ratio when the PAR level is increased. PIDyn increased in H_{PAR} as a photoprotection mechanism, since a reduction in the reversible PSII quantum potential yield (F_v/F_m) was found, accompanied by a significant increase in the thermal dissipation of excess absorbed energy, these processes being photoprotection mechanisms [8]. The above reduces the effect on proteins of the photosynthetic apparatus [62]. Finally, cocoa genotypes under H_{PAR} showed a decrease in Chl_t; in parallel, they also showed a reduction in the *Chl*/*Car* ratio, which favors the dissipation of excess energy in the form of heat. However, increased MDA concentration and electrolyte leakage were found to mean more damage at the cellular level in H_{PAR}.

Our study mainly found that cocoa plants exhibit optimal physiological behavior under low radiation conditions, specifically at mean PAR levels of 400 µmol m^{-2} $s^{-1,}$ which different studies have reported. However, this finding is contrary to what has been reported by Suárez et al. [6] for the same study area. In that study, cocoa plants show an optimal physiological performance under H_{PAR} conditions, mainly attributed to the high cloud cover typical of the Colombian Amazon, a region that presents an annual average of only 3–4 h of daily radiation [33]. These differences are also probably due to the state of development of the cocoa plants. The plants we used in our study had 13 months of development while those used by Suárez et al. [6,63] report a growth of 36 months (productive phase) under agroforestry systems with different levels of radiation transmitted. Therefore, the time difference between these two experiments that were conducted at the same site was 23 months, demonstrating the sensitivity to radiation at the early stages of development. When we compare the biochemical responses reported by Suárez et al. [6] as a function of carotenoid concentration and chlorophyll a/b ratio with those obtained in the present study, we observe marked differences specifically for genotype CCN51, which was evaluated in both studies. In this sense, it has been reported that the variation in carotenoid content can be considered an adjustment or mechanism of photoprotection, which is related to growth time and environmental conditions [64,65].

Different studies report that variability in carbon uptake may be due to differences in age and growing conditions [20–28]. Since there is no universal agreement on the exact amount of shade required to maximize cocoa production, our results are of utmost importance, specifically because they show how different cocoa genotypes cope with increased radiation, supported by analysis of the relationship with water, diffusive components and biochemicals at leaf level.

Therefore, based on the results obtained by Suárez et al. [6] and Jaimez et al. [29] in areas where most of the year there is cloud cover and low air evaporation demand, the importance of our study lies in the different responses of cocoa to light in field conditions when it is cloudy. These data are very important when deciding which genotype is best adapted to the Colombian Amazon conditions [6], since it is currently considered the "crop for peace", that is, a crop to replace illegal farming systems. In this sense, there is the possibility of growing cocoa with a low level of shade and taking advantage of the availability of cocoa materials that have high radiation acclimatization. In this study, we

highlight genotypes such as ICS-1 and LUK-40 that exhibit physiological and biochemical mechanisms to maintain the balance between the use of energy and/or dissipation capacity, thus maintaining carbon fixation. Taken together, these facts suggest that, under the conditions of the Colombian Amazon, some cocoa genotypes have the ability to acclimatize to high levels of light as long as they are under high cloudiness conditions.

5. Conclusions

This study shows the physiological behavior of different cocoa genotypes under contrasting radiation levels, being superior at a low radiation level for the ICS-95 and LUK-40 genotypes. However, even more importantly, it is possible to demonstrate that these same cocoa genotypes exhibit improved carbon assimilation performance, demonstrating photosynthetic acclimatization to high solar radiation patterns that resulted in higher A_{max} and V_{cmax}; this was possible to the high cloud cover conditions prevailing in the Colombian Amazon. Our results also suggest the need to expand the analysis of adaptation to other universal and regional cocoa genotypes in order to find materials that demonstrate greater photosynthetic acclimatization.

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