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Research article

Genetic variation in high light responses of *Theobroma cacao* L. accessions

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ABSTRACT

Cacao (*Theobroma cacao* L.) is a shade-tolerant tree species, but in recent years it has increasingly been cultivated under full sun conditions in an orchard system where photoinhibition is likely. Here we investigate the extent of photoinhibition in 17 cacao accessions from a range of genetic groups, growing under high light conditions. The ability of the photosynthetic systems to respond to high light was assessed using chlorophyll fluorescence parameters (diurnal F_v/F_m and instantaneous light response curves), and differences in photosynthetic pigment content were compared using biochemical assays. Damage due to photoinhibition was assessed using electrolyte leakage, lipid peroxidation, and reactive oxygen species scavenging systems were compared using biochemical assays (for APX, CAT and SOD). There was significant variation between the 17 accessions for photosynthetic parameters, although in all cases the light saturation points were well below the midday light levels. Light acclimation of photosynthetic pigments was evident and variation in the total chlorophyll to total carotenoid ratio was significantly correlated with electrolyte leakage. Significant genetic variation was observed across the 17 accessions in the activities of CAT, APX and SOD. Across all accessions, photoprotection appeared to be restricted by the ability of leaves to generate SOD. Significant negative correlations were observed between SOD activity and both APX activity and electrolyte leakage, while significant positive correlations were observed between electrolyte leakage and both APX and CAT activity. Accessions with higher light saturation points, as well as high carotenoid and high SOD concentrations were able to tolerate the moderately high light, however, none of the accessions were clearly superior to the commonly grown Amelonado accession. The results imply that screening for SOD activity, total carotenoid content and light saturation point can aid in selection of genotypes with better tolerance to high light.

1. Introduction

Cacao (*Theobroma cacao* L.) is a tropical shade tolerant tree that originated in the Upper Amazon (Motamayor et al., 2002) where it grows in the understorey of larger trees (Daymond et al., 2011). Cacao trees are grown in tropical regions including South America, West Africa and South-East Asia (Farrell et al., 2018; Lahive et al., 2019). Cacao beans are exported mainly to North America and Europe (Clough et al., 2009) for use in the production of chocolate and confectionery (Lahive et al., 2019).

Cacao can be grown using either an agroforestry system where cacao trees are associated with other trees which provide shade (Clough et al., 2009; Jagoret et al., 2017), or an intensive system using selected varieties that are managed under minimal homogenous shade or without shade (Jagoret et al., 2017; Neither et al., 2020). Though in general the practice has been to grow cacao under shade there is now a trend towards a monoculture system lacking shade trees, due to the potential for increased yields (Zuidema et al., 2005; Ruf et al., 2010; Lahive et al., 2019), although not necessarily increased profitability (Neither et al., 2020). However, it is not known if the currently available cacao accessions are able to tolerate high light intensities common in these full sun systems.

When exposed to high photosynthetic photon flux density (PPFD), plants must be able to both utilize the light energy and ensure that damage to the photosystems is avoided. When the absorption of light energy exceeds the capacity for photoquenching, the primary electron acceptor of photosystem II (PSII) namely Q_A becomes overly reduced and the potential to generate reactive oxygen species (ROS) is increased (Dietz et al., 2016). Excess light energy beyond that which can be used in photochemical quenching may result in either reversible photoinhibition of PSII or chronic photoinhibition including longer term damage to photosystem I (PSI) (Allen and Ort, 2001; Osmond and Förster, 2008)

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both of which results in a reduction in the productivity of the plant (Huner et al., 1998; Simkin et al., 2017; Lima-Melo et al., 2019). Photoinhibition occurs frequently in plants growing in tropical regions as light intensity can be as high as 1800 μmol m^{-2} s^{-1} of PPFD (Larcher, 1995; Krause et al., 2001).

For effective protection against excess irradiance, it is essential for plants to employ mechanisms that dissipate light energy (Foyer, 2018). These mechanisms are crucial for the regulation of the photosynthetic apparatus and allow the plant to continuously adjust to changes in light intensity (Yokthongwattana and Melis, 2006). These mechanisms allow energy-producing processes to continue whilst protecting PSII and PSI from light-induced oxidative inactivation (Ruban et al., 2012; Aro et al., 1993; Yokthongwattana and Melis, 2006). Absorbed light energy can be dissipated by the nonphotochemical quenching (NPQ) processes namely PsbS protonation, state transition quenching, photoinhibitory quenching and zeaxanthin formation in the xanthophyll cycle (Cupellini et al., 2020; Demmig-Adams et al., 1996; Mathur et al., 2018; Sukhova et al., 2020; Szymańska et al., 2017). Alongside these NPQ processes, photochemical quenching can dissipate excess energy by maintaining electron flow through the photosynthetic apparatus by using other routes such as cyclic election flow around PSI, the Mehler reaction and photorespiration (Mathur et al., 2018; Szymańska et al., 2017; Ort and Baker, 2002). These protective processes can however lead to an increase in the generation of ROS. When these protective mechanisms are overwhelmed, ROS are produced in the chloroplasts which can lead to oxidative damage of cell components. This results in electrolyte leakage through K^+ channels and membrane damage which can lead to metabolic adjustment for adaption and repair processes. ROS generation under extreme stress can result in the triggering of programmed cell death (Demidchik et al., 2014; Mittler, 2017).

To overcome problems associated with ROS generation, plants contain complex enzymatic and antioxidant systems which mitigate the effects of excess light energy and other stresses (Martinez et al., 2001; Meloni at al., 2003; Szymańska et al., 2017; Dumanovic et al., 2021). The enzymes in this system include superoxide dismutase (SOD; EC 1. 15.1.1), catalase (CAT; EC 1.11.1.6) and ascorbate peroxidase (APX; EC 1.11.1.11). SOD is considered the first line of defence against O_2^- which it converts to H_2O_2. CAT and APX both function downstream of SOD detoxifying H_2O_2 converting it to O_2 (Asada, 1999; Dumanovic et al., 2021).

The ability to respond to high light varies between plant species, and it is important to understand a species relative sensitivity and potential for acclimation (Krause et al., 2001). The characteristics of shade-grown plants that render them more susceptible to light, drought and heat stress when suddenly exposed to high irradiance includes a high concentration of chlorophyll per leaf area, a greater capacity for light capture due to the larger antenna size of PSII with less reaction centres and lower light-saturated photosynthetic rates due to reduced Calvin cycle photosynthetic enzymes content (Lovelock et al., 1994; Mathur et al., 2018; Osmond, 1994; Wu et al., 2020).

The maximum photosynthetic rate of cacao leaves occurs under low light intensities, with light saturation points of below 400 μmol m^{-2} s^{-1} being reported by a number of studies (Raja Harun and Hardwick, 1988; Baligar et al., 2008; Daymond et al., 2011; Lennon et al., 2021). Raja Harun and Hardwick (1988) demonstrated that cacao leaves exposed to light levels above 500 μmol m^{-2} s^{-1} underwent a rapid reduction in their photosynthetic rate, leading to photoinhibition. This work also demonstrated a linear decrease in photosynthetic rate with exposure time at light intensities between 200 and 800 μmol m^{-2} s^{-1} with the rate of decline being more rapid at higher irradiances. A study by Daymond et al. (2011) reported genotypic variation in both net photosynthetic rate and quantum efficiency, however their study was limited to eight genotypes.

As cacao is an understory species, it is expected to have only a limited potential for acclimation to high light. There have been few studies carried out in cacao to investigate protective mechanisms against ROS production due to high light intensity (Lennon et al., 2021) with no studies investigating genotypic variation. This study seeks to determine genetic variation in photosynthetic responses and protective mechanisms under high light, by comparing the responses of 17 accessions representing a wide genetic background. This study aims to identify valuable biomarkers that can be used to screen different accessions for high light tolerance which could be used to implement a cocoa plant breeding programme to produce plants with desirable agronomic traits.

2. Materials and methods

2.1. Plant material and experimental design

The study was conducted at the University of the West Indies, St. Augustine Campus, Trinidad and Tobago. Seventeen, 6 ½ -year-old *Theobroma cacao* accessions were selected for this study with three replicates, the accessions with the corresponding genetic groups are shown in Table 1. The plants were grown in 22 L pots using 2 parts soil, 1 part course sand and 1 part cured manure and completely randomized under experimental conditions of moderately high light (30% shade). The experiment was conducted under shadehouse conditions with mean light intensity values (measured on days when photosynthetic measurements were taken, n = 9) of 400 μmol m^{-2} s^{-1} at 9:00 h, 700 μmol m^{-2} s^{-1} at 12:00 h and 425 μmol m^{-2} s^{-1} at 16:00 h (PPFD, measured with a Li-250A, Licor Biosciences, NA, USA). All 51 plants (3 plants per accession) were sprayed with insecticides, together with a foliar fertiliser every fortnight and watered daily. All experimentation was performed on upper canopy leaves of the interflush 2 developmental stage, representing the beginning of lignification of the petioles (Greathouse et al., 1971; Snoeck, 1987).

2.2. Measurement of photosynthetic parameters

Chlorophyll fluorescence was measured using a MINI-PAM portable chlorophyll fluorometer (Walz, Effeltrich, Germany) on attached leaves. The manufactures 'burst mode' was utilised so that fluorescence is excited by pulse modulated red light from a bespoke light-emitting-diode (LED; passed through a cut-off filter to produce an excitation band peaking at 650 nm, with negligible wavelengths beyond 700 nm), a pulse-width of 0.2 s is alternated with 0.8 s dark-intervals. The settings were optimised for use with dark-adaptation clips with measuring light set to 60 μmol m^{-2} s^{-1}, actinic light set to 100 μmol m^{-2} s^{-1} (duration: 30 s) and saturating pulse intensity set to 10,000 μmol m^{-2} s^{-1} (duration: 800 ms).

The maximum (F_m) and basal (F_o) fluorescence yields were determined after 30 min of dark adaption and used to determine the maximum potential quantum efficiency of PSII (F_v/F_m) calculated as $F_v/F_m = (F_m\text{-}F_o)/F_m$ with the leaves sampled on the same day at 9:00 h, 12:00 h and 16:00 h. For the various measurements three leaves were sampled per tree, with three replicates over time (n = 9). Instantaneous light response curves were obtained using the MINI-PAM programme with a 30 s interval at each light level. This employs the built in halogen lamp with heat-reflecting and short-pass filters producing a white light with negligible wavelengths beyond 700 nm. The eight light intensities were measured directly within the leaf-clip with a light meter (Li-250A, Licor Biosciences, NA, USA) and set to produce approximately 60, 120, 180, 240, 400, 500, 750, and 1250 μmol m^{-2} s^{-1} PPFD at the leaf surface.

Light response curves data were used to determine the Quantum Yield, maximum electron transport rate (ETR_{max}) and saturating photosynthetic photon flux density ($PPFD_{sat}$). The light saturation point was determined for each leaf by fitting a simple exponential decay function (Ritchie, 2008) and visually inspecting each curve to determine the first point where the saturation plateau is reached.

The PSII operating efficiency at each light level was determined by $F_q'/F_m' = F_m'\text{-}F/F_m'$, where F is steady-state fluorescence in the light and F_m' is maximum fluorescence in the light when saturating light is imposed (Baker, 2008). The apparent photosynthetic electron transport rate (ETR)

was calculated as F_q'/F_m' x PPFD x 0.5 × 0.84. The factor 0.84 accounts for light that is not absorbed by either photosystem and assumes that 16% of PPFD is reflected. The factor 0.5 accounts for the excitation of both PS II and PS I and makes the assumption of equal absorbance by both photosystems (Rascher et al., 2000).

2.3. Pigment analysis

For each leaf, two 1.7 cm diameter leaf discs were taken, ground in 5 mL of 80 % acetone and the homogenate centrifuged at 20,000 x *g* for 10 min. The pellet was re-extracted with an additional 5 mL of 80 % acetone and centrifuged at 20,000 x *g* for 10 min. Chlorophyll *a* (c_a), chlorophyll *b* (c_b) and total carotenoid ($c_{(x+c)}$) content were measured spectrophotometrically using a Thermos Scientific Genesys 10S UV-VIS Spectrophotometer and determined according to Lichtenthaler and Buschmann (2001).

2.4. Extraction and determination of enzyme activities

For each leaf, ten 1.7 cm diameter leaf discs, were ground on ice in 5 mL of cold extraction buffer (50 mM K_2PO_4 (pH 7), 2 mM EDTA, 20 mM ascorbic acid, 0.1 % (v/v) Triton X-100 and 2 % (w/v) PVP). Following centrifugation at 17,000 x *g* for 30 min at 4 °C the supernatant was retained for enzyme analysis.

The activity of SOD was assayed using the method described by Lima et al. (2002) by following the inhibition the photochemical reduction of nitrobluetetrazolium (NBT) as described by Lima et al. (2002). 3 mL reactions containing 50 mM NaH_2PO_4 (pH 7.8), 14 mM methionine, 0.2 mM EDTA, 75 μM NBT, 0.25 mM riboflavin and 50 μL enzyme extract, alongside a control reaction without the enzyme extract, were prepared. The assay was started by illuminating the samples in a foil lined box using a 15 W fluorescent light. Duplicate blank reactions were also prepared and incubated in the dark. A 50 % inhibition of NBT reduction represents one enzyme unit.

APX activity was measured as described by Lima et al. (2002) using a 3 mL reaction volume containing 100 mM K_2PO_4 (pH 7), 1 mM H_2O_2, 0.5 mM ascorbic acid and 50 μL enzyme extract. Enzyme activity was measured using a Thermos Scientific Genesys 10S UV-VIS Spectrophotometer by following the decrease in absorbance at 290 nm with one enzyme unit being defined as the ability to oxidize 1 μmol ascorbate min^{-1}.

The activity of CAT was measured as described by Lima et al. (2002) in a 3 mL reaction volume containing 50 mM K_2PO_4 (pH 7), 12.5 mM H_2O_2 and 50 μL enzyme extract. Activity was measured using a Thermos Scientific Genesys 10S UV-VIS Spectrophotometer by following the reduction in absorbance at 240 nm. One enzyme unit is defined as the amount of enzyme able to breakdown 1 μmol H_2O_2 min^{-1}.

2.5. Electrolyte leakage

For each leaf, twenty-five 1.7 cm diameter leaf discs were washed three times with distilled water followed by incubation in deionized water for 24 h at room temperature in the dark using an incubator shaker at 2,000 x *g*. Conductivity was recorded using a OAKTON PC 700 (Oakton Instruments IL.) meter. The samples were then lysed by autoclaving at 120 °C/0.10 MPa for 30 min following which conductivity was again recorded. Percentage ion leakage was calculated as: (Pre-lysis conductivity/Post-lysis conductivity) x 100 (Sunkar, 2010).

2.6. Lipid peroxidation assay

For each leaf, one 1.7 cm diameter leaf disc was homogenized in 3 mL of 0.1 % TCA. The extract was centrifuged at 13,000 x *g* for 2 min then 0.5 mL of supernatant was added to 1.5 mL of Thiobarbituric acid (TBA; 0.5 % in 20 % TCA). The samples were incubated at 90 °C for 20 min followed by rapid cooling. Following centrifugation at 13,000 x *g* for 5 min the supernatant was retained. Absorbance was measured spectrophotometrically at 532 nm and nonspecific turbidity was corrected for by subtracting the absorbance at 600 nm. MDA concentration was calculated using its extinction coefficient (155 mM^{-1} cm^{-1}) (Sunkar, 2010).

Table 1. List of accessions of *Theobroma cacao* and their corresponding genetic groups used in this study.

Genetic Group	Accession
Amelonado	AM 2/6
Contamana	SCA 6
Curaray	LCT EEN 68/S2
Guiana	GU 310
Iquitos	B 18/5
Marañon	PA 169, PA 279, PA 289, PA 296
Nacional	NA 471
Nanay	CRU 100
Retractario	CL 27/50, CLM 100, LP 1/21, MOQ 2/10, LV 20
Trinitario	ICS 1

2.7. Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) using SPSS software (SPSS Inc. IL. 2008). For diurnal data, analysis was carried out using one-way ANOVA with the time of day (9:00, 12:00 or 16:00) as fixed factors. One-way ANOVA with the light level as a fixed factor was used to analyse the light response curves. Pearson correlations (Row-wise deletion) were analysed using NCSS software (NCSS 11 Statistical Software. UT. 2016).

3. Results

3.1. Photosynthetic parameters

Significant differences ($P \leq 0.05$) were observed between accessions in the maximum photochemical efficiency of PSII (F_v/F_m). At 9:00 h, F_v/F_m values ranged from 0.702 ± 0.013 (PA 169) to 0.812 ± 0.013 (LCT EEN 68/S2) representing a 0.16-fold difference between the lowest and highest values (Figure 1).

Evaluation of the plants at 12:00 h showed that F_v/F_m was lower when compared to the values at 9:00 h, however, no significant differences ($P > 0.05$) were observed in the percentage reduction (from 9:00 to 12:00 h). At 12:00 h, F_v/F_m values ranged from 0.674 ± 0.019 (LV 20) to 0.765 ± 0.019 (AM 2/6) representing a 0.14-fold difference between the lowest and highest values. At 16:00h, F_v/F_m values ranged from 0.701 ± 0.015 (LV 20) to 0.813 ± 0.015 (B 18/5) representing a 0.16-fold difference between the lowest and highest values (Figure 1). In general all accessions displayed some level of recovery of the F_v/F_m values overnight, however there were no significant differences in the percentage recovery (from 12:00 to 16:00 h) amongst the accessions. A number of accessions namely PA 169, PA 269, NA 471, MOQ 2/10 and LV 20 had F_v/F_m values that remained below 0.8 at all time points measured. The F_v/F_m values did not display significant correlations with the other photosynthetic measurements made in this study.

Significant differences ($P \leq 0.05$) were observed between accessions in maximum Electron Transport Rate, Light Saturation Point and Quantum Yield (Figure 2). Electron Transport Rates varied between 23.33 ± 1.92 μmol m^{-2} s^{-1} (SCA 6) to 38.33 ± 1.92 μmol m^{-2} s^{-1} (B 18/5) representing a 0.64-fold difference between the lowest and highest values. Saturating irradiance ranged from 173.33 ± 24.17 μmol m^{-2} s^{-1} (PA 296) to 326 ± 24.17 μmol m^{-2} s^{-1} (PA 169) representing a 0.88-fold difference between the lowest and highest values. Quantum yield ranged from 0.10 ± 1.04 × 10^{-2} (SCA 6) to 0.17 ± 1.04 × 10^{-2} (B 18/5) representing a 0.7-fold difference between the lowest and highest values.

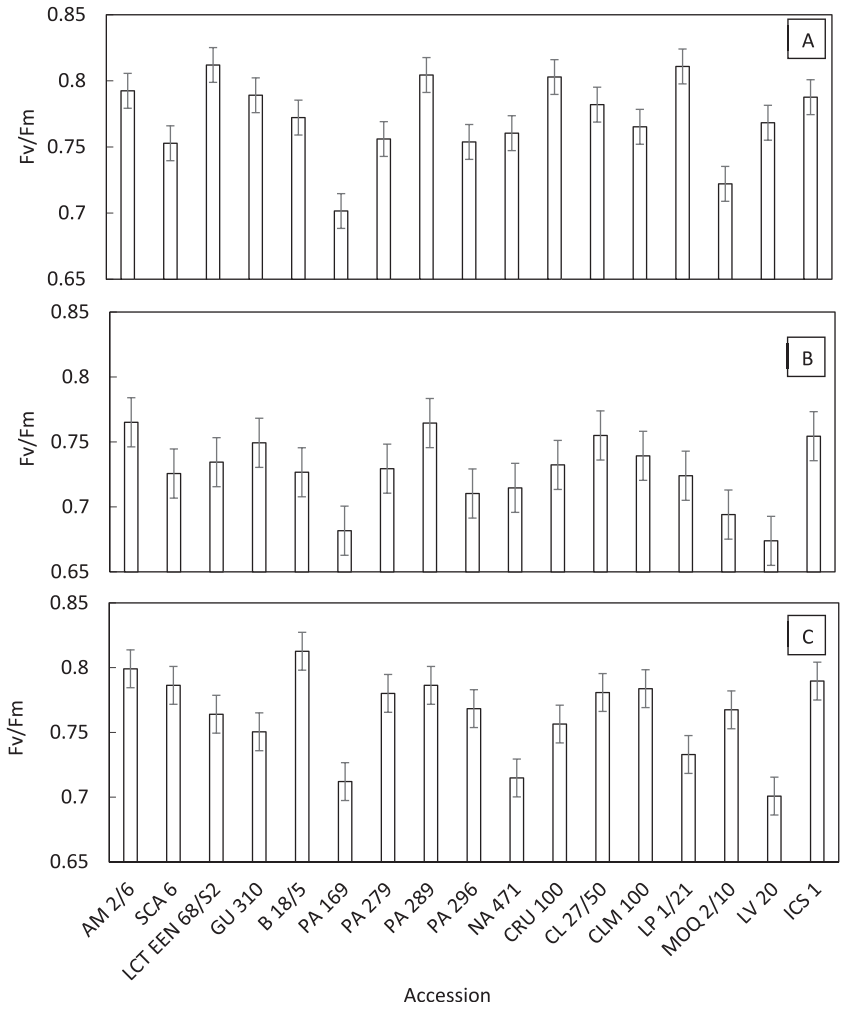

Figure 1. Genetic variation in ratio of variable fluorescence to maximal fluorescence (F_v/F_m) at (A) 9:00 h, (B) 12:00 h and (C) 16:00 h of seventeen cacao genotypes grown under high light conditions. Mean comparisons were performed using ANOVA at $P \leq 0.05$. Bars represent mean ± SEM (n = 9), measured over a period of nine days.

The Electron Transport Rate had significant strong correlations with both $PPFD_{sat}$ (0.685) and Quantum yield (0.957) (Table 3).

3.2. Pigment analysis

Significant differences were observed ($P \leq 0.05$) in photosynthetic pigments between the 17 accessions (Table 2). Chlorophyll *a* values ranged from 1.146 ± 0.209 mg g^{-1} DW (PA 169) to 2.879 ± 0.209 mg g^{-1} DW (CRU 100) representing a 1.51-fold difference between the lowest and highest values. Chlorophyll *b* values ranged from 0.385 ± 7.329×10^{-2} mg g^{-1} DW (PA 169) to 1.320 ± 7.329×10^{-2} mg g^{-1} DW (LV 20) representing a 2.43-fold difference between the lowest and highest values. Total chlorophyll values ranged from 1.531 ± 0.273 mg g^{-1} DW (PA 169) to 3.774 ± 0.273 mg g^{-1} DW (CRU 100) representing a 1.47-fold difference between the lowest and highest values. Total carotenoid values ranged from 0.412 ± 0.053 mg g^{-1} DW (PA 169) to 0.909 ± 0.053 mg g^{-1} DW (CRU 100) representing a 1.21-fold difference between the lowest and highest values.

The ratio of chlorophyll *a* to chlorophyll *b* ($c_{a/b}$) and chlorophylls *a* and *b* to total carotenoids ($c_{(a+b)}/c_{(x+c)}$) showed significant differences ($P \leq 0.05$) (Figure 3). The chlorophyll *a* to chlorophyll *b* ratio ranged from 1.807 ± 0.123 (LV 20) to 3.172 ± 0.123 (CRU 100) representing a 0.76-fold difference between the lowest and highest values. The total carotenoids ratio ranged from 3.635 ± 0.164 (PA 169) to 5.26 ± 0.164 (LV 20) representing a 0.45-fold difference between the lowest and highest values.

3.3. Enzyme activities

Significant differences ($P \leq 0.05$) were observed between accessions in enzyme activity (Figure 4). SOD activity values ranged from 1287.86 ± 96.38 U g^{-1} DW (CL 27/50) to 1982 ± 96.38 U g^{-1} DW (AM 2/6) representing a 0.54-fold difference between the lowest and highest values.

The values for APX ranged from 42.59 ± 8.91 U g^{-1} DW (PA 169) to 128.43 ± 8.91 U g^{-1} DW (MOQ 2/10) representing a 2-fold difference between the lowest and highest values. CAT activity ranged from 2.86 ± 0.45 U g^{-1} DW (PA 169) to 5.01 ± 0.45 U g^{-1} DW (MOQ 2/10) representing a 0.75-fold difference between the lowest and highest values. APX activity had significant ($P \leq 0.05$) moderate positive correlation with CAT activity (0.667) and a significant ($P \leq 0.05$) moderate negative correlation with SOD activity (-0.513) (Table 3).

3.4. Electrolyte leakage

Significant differences ($P \leq 0.05$) were observed between accessions in electrolyte leakage. The values ranged from 12.03% ± 0.486 (AM 2/6) to 16.65% ± 0.486 (CRU 100) representing a 0.38-fold difference between the lowest and highest values (Figure 5). Electrolyte leakage had a significant ($P \leq 0.05$) moderate negative correlation (-0.538) with the total chlorophyll to total carotenoid ratio. Electrolyte leakage also displayed a significant positive moderate correlation (0.594) to APX activity and a significant strong negative correlation (-0.961) with SOD activity.

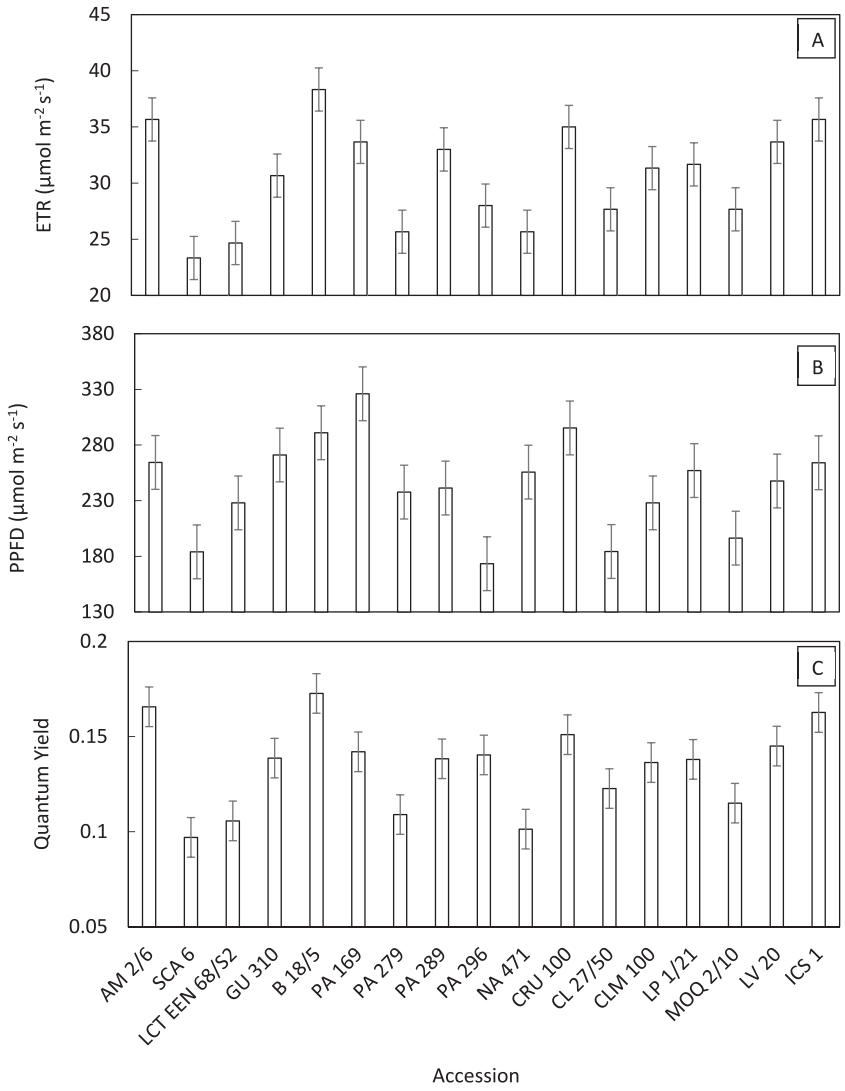

Figure 2. Genetic variation in the (A) maximum Electron Transport Rate, (B) Light Saturation point and (C) Quantum Yield of seventeen cacao genotypes grown under high light conditions. Mean comparisons were performed using ANOVA at $P \leq 0.05$. Bars represent mean ± SEM ($n = 9$).

3.5. Lipid peroxidation

Significant differences ($P \leq 0.05$) in MDA content were observed between accessions in lipid peroxidation. The values ranged from 5.52 ± 0.43 µg/g DW (CL 27/50) to 8.01 ± 0.3 µg/g DW (NA 471) representing a 0.45-fold difference between the lowest and highest values (Figure 6). Lipid peroxidation did not display any significant correlations with the other parameters measured in this study.

4. Discussion

All of the cacao accessions showed the responses expected for an understory species, with none of the accession taking full advantage of the high light conditions. In general, to be well adapted to high light intensity under an orchard system cocoa accessions would require the ability to avoid photoinhibition by having the capacity to quench light energy absorbed alongside the ability to mitigate ROS production through the activity of scavenging enzymes.

The F_v/F_m ratio represents the maximum potential quantum efficiency of PSII if all reaction centres are open (Baker, 2008). A value of 0.83 is considered optimal for most plant species (Maxwell and Johnson, 2000) and values lower than 0.83 are obtained when a plant is under stress with these lower values possibly representing photoinhibition (Baker, 2008). The accessions used for this study all had F_v/F_m values below 0.83 at 9.00 h and lower values were obtained in all accessions at the noon reading, ranging between 0.67 and 0.77, indicating increased stress to the tissue as the light levels increased from 9.00 to 12.00 h. By 16:00 h all of the accessions had demonstrated some level of recovery indicated by increases in the F_v/F_m values obtained and with some level of recovery continuing overnight. The range of values obtained indicated that there is genetic variation in the sensitivity of PSII with some accessions e.g., AM 2/6 and PA 289 displaying reduced tissue stress and showing recovery by 16.00h suggesting reversible photoinhibition. Other accessions e.g., PA 169 and LV 20 with low F_v/F_m values at all-time points examined may represent chronic photoinhibition (Maxwell and Johnson, 2000). Work by Acheampong et al. (2013) using four cocoa clones demonstrated a reduction in the F_v/F_m ratio between 8:00 to 10:00 h with a recovery occurring between 10:00 to 16:00 h. The observed recovery was to values equal to or higher than those seen at 8:00 h. Variation in the response of clones was observed in particular under low shade regimes and genetic variability was reported with PA 150 and SCA 6 showing greater levels of photoinhibition compared to the other accessions used in the study. Lennon et al. (2021) demonstrated that full sun leaves in the accession WAA showed inhibition during the day, with recovery only occurring overnight, whereas shade leaves showed no evidence of photoinhibition. These studies alongside the current one all demonstrate that cocoa is

Table 2. Genetic variation in content of chlorophyll *a* (c_a), chlorophyll *b* (c_b), total chlorophylls ($c_{(a+b)}$) and total carotenoids ($c_{(x+c)}$) of seventeen cacao genotypes. Sampling was carried out at 9:00 h.

Accession	c_a (mg g^{-1}DW)	c_b (mg g^{-1}DW)	$c_{(a+b)}$ (mg g^{-1}DW)	$c_{(x+c)}$ (mg g^{-1}DW)
AM 2/6	2.369 ± 0.209	0.868 ± 7.329 × 10^{-2}	3.237 ± 0.273	0.728 ± 0.053
SCA 6	2.303 ± 0.209	0.771 ± 7.329 × 10^{-2}	3.074 ± 0.273	0.761 ± 0.053
LCT EEN 68/S2	1.986 ± 0.209	0.665 ± 7.329 × 10^{-2}	2.651 ± 0.273	0.635 ± 0.053
GU 310	2.312 ± 0.209	0.834 ± 7.329 × 10^{-2}	3.146 ± 0.273	0.774 ± 0.053
B 18/5	2.788 ± 0.209	0.904 ± 7.329 × 10^{-2}	3.692 ± 0.273	0.847 ± 0.053
PA 169	1.146 ± 0.209	0.385 ± 7.329 × 10^{-2}	1.531 ± 0.273	0.412 ± 0.053
PA 279	1.600 ± 0.209	0.600 ± 7.329 × 10^{-2}	2.201 ± 0.273	0.529 ± 0.053
PA 289	2.012 ± 0.209	0.744 ± 7.329 × 10^{-2}	2.756 ± 0.273	0.606 ± 0.053
PA 296	2.320 ± 0.209	0.784 ± 7.329 × 10^{-2}	3.105 ± 0.273	0.773 ± 0.053
NA 471	2.241 ± 0.209	0.757 ± 7.329 × 10^{-2}	2.999 ± 0.273	0.715 ± 0.053
CRU 100	2.879 ± 0.209	0.894 ± 7.329 × 10^{-2}	3.774 ± 0.273	0.909 ± 0.053
CL 27/50	2.456 ± 0.209	0.861 ± 7.329 × 10^{-2}	3.318 ± 0.273	0.761 ± 0.053
CLM 100	1.749 ± 0.209	0.592 ± 7.329 × 10^{-2}	2.341 ± 0.273	0.561 ± 0.053
LP 1/21	2.230 ± 0.209	0.749 ± 7.329 × 10^{-2}	2.979 ± 0.273	0.723 ± 0.053
MOQ 2/10	1.809 ± 0.209	0.640 ± 7.329 × 10^{-2}	2.449 ± 0.273	0.593 ± 0.053
LV 20	2.390 ± 0.209	1.320 ± 7.329 × 10^{-2}	3.710 ± 0.273	0.706 ± 0.053
ICS 1	2.219 ± 0.209	0.757 ± 7.329 × 10^{-2}	2.976 ± 0.273	0.699 ± 0.053

Mean comparisons were performed using ANOVA at $P \leq 0.05$. Values are expressed as mean ± SEM (n = 6).

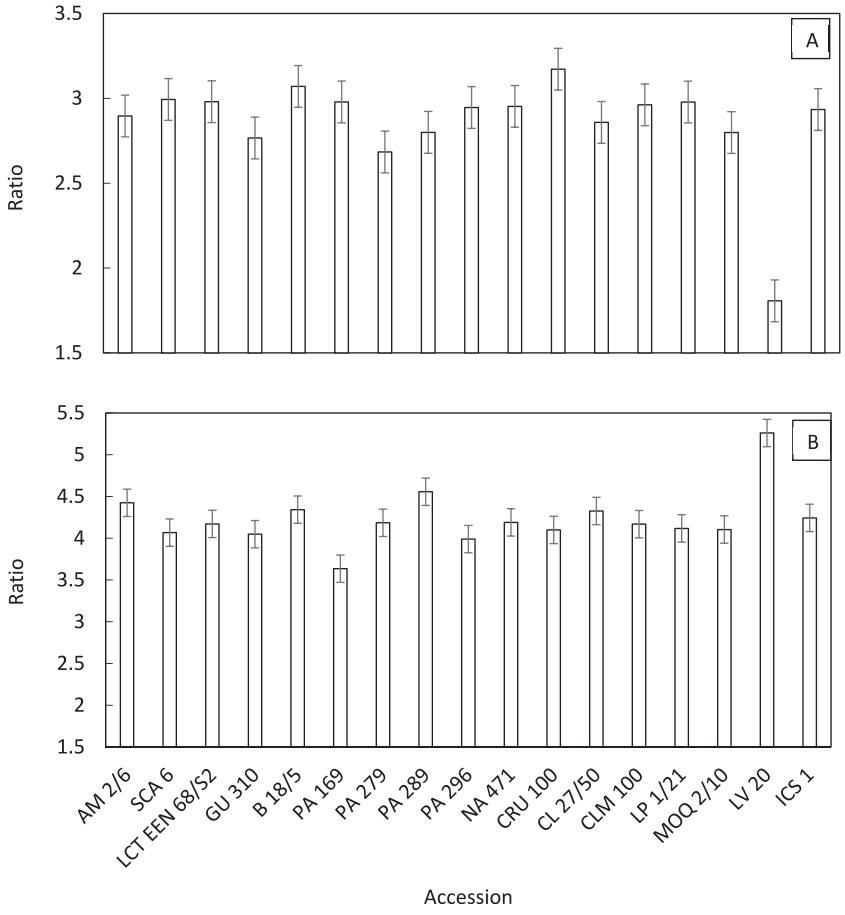

Figure 3. Genetic variation in (A) ratio of chlorophyll *a* to chlorophyll *b* ($c_{a/b}$) and (B) chlorophylls *a* and *b* to total carotenoids ($c_{(a+b)}/c_{(x+c)}$) of leaves from seventeen cacao genotypes grown under high light conditions. Mean comparisons were performed using ANOVA at $P \leq 0.05$. Bars represent mean ± SEM (n = 6).

susceptible to photoinhibition but there is genetic variation in the extent of photoinhibition each accession experiences. The level of photoinhibition also appears to be dependent on the light regime under which the plants are grown.

Genetic variation was also observed in the other photosynthetic parameters measured in this study. However, in all cases the light saturation point of the accessions was below the PPFD measured at all the time points investigated, suggesting that the cocoa accessions used

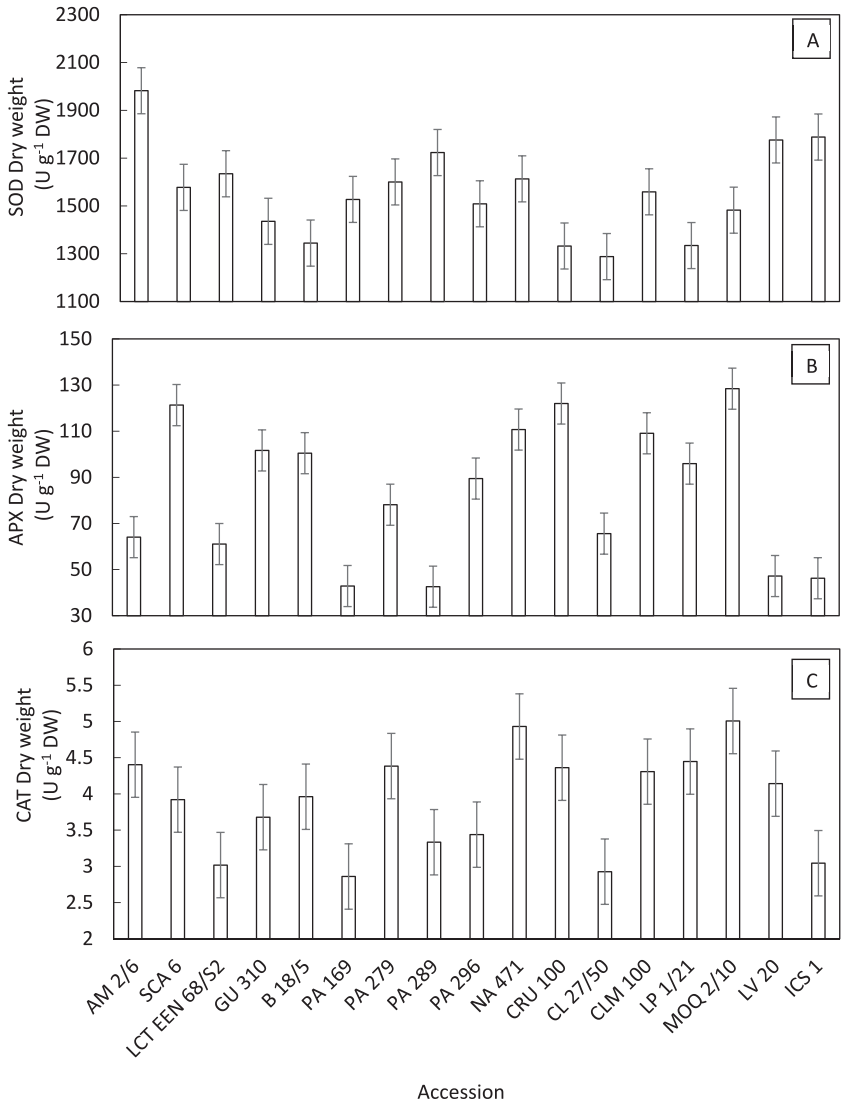

Figure 4. Genetic variation in (A) superoxide dismutase-SOD, (B) ascorbate peroxidase-APX and (C) catalase-CAT activities in seventeen cacao genotypes grown under high light conditions. Mean comparisons were performed using ANOVA at $P \leq 0.05$. Bars represent mean ± SEM (n = 6).

Table 3. Correlation matrix of pearson correlation coefficients.

	$c_{(a+b)}/c_{(x+c)}$	F_v/F_m at 12h	ETR_{max}	$PPFD_{sat}$	QY	SOD	APX	CAT	Elec Leak
$c_{(a+b)}/c_{(x+c)}$	1	-0.013	0.252	-0.093	0.209	0.421	-0.345	0.145	-0.538*
F_v/F_m at 12h	-0.013	1	0.115	-0.065	0.183	0.137	-0.101	-0.217	-0.132
ETR_{max}	0.252	0.115	1	0.685**	0.957***	0.109	-0.296	-0.077	-0.175
$PPFD_{sat}$	-0.093	-0.065	0.685**	1	0.543*	0.035	-0.224	-0.019	-0.030
QY	0.209	0.183	0.957***	0.543*	1	0.114	-0.295	-0.155	-0.174
SOD	0.421	0.137	0.109	0.035	0.114	1	-0.513*	0.003	-0.961***
APX	-0.345	-0.101	-0.296	-0.224	-0.295	-0.513*	1	0.667**	0.599*
CAT	0.145	-0.217	-0.077	-0.019	-0.155	0.003	0.667**	1	0.134
Elec Leak	-0.538*	-0.132	-0.175	-0.030	-0.174	-0.961***	0.599*	0.134	1

* significant at $p \leq 0.05$; ** significant at $p \leq 0.005$; *** significant at $p < 0.001$.

in this study were unable to utilize PPFD beyond 300 μmol m^{-2} s^{-1}. These results are similar to other studies (Baligar et al., 2008; Daymond et al., 2011; Suárez Salazar, 2018: Lennon et al., 2021) with the specific saturation point varying with the accession used and the light regime under which the cocoa accessions were grown. When comparing these photosynthetic parameters between accessions, it is important to consider variation in leaf traits that may influence the estimation of ETR due to differences in the fraction of light absorbed or the distribution of light between PSI and PSII (Sukhova et al., 2018, 2020). Nonetheless, instantaneous light response curves provide a robust tool for comparing responses between accessions, particularly where multiple parameters are assessed (Ritchie 2008). Our study showed strong positive

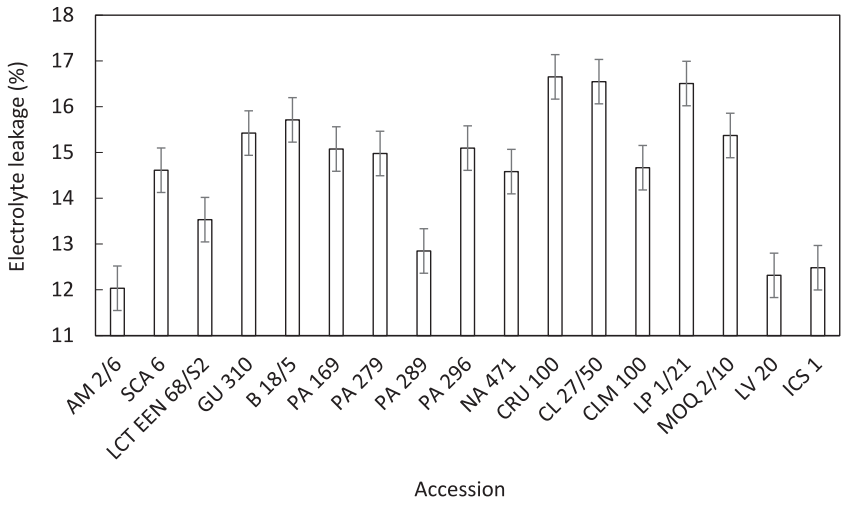

Figure 5. Genetic variation in electrolyte leakage of seventeen cacao genotypes grown under high light conditions. Mean comparisons were performed using ANOVA at $P \leq 0.05$. Bars represent mean ± SEM (n = 6).

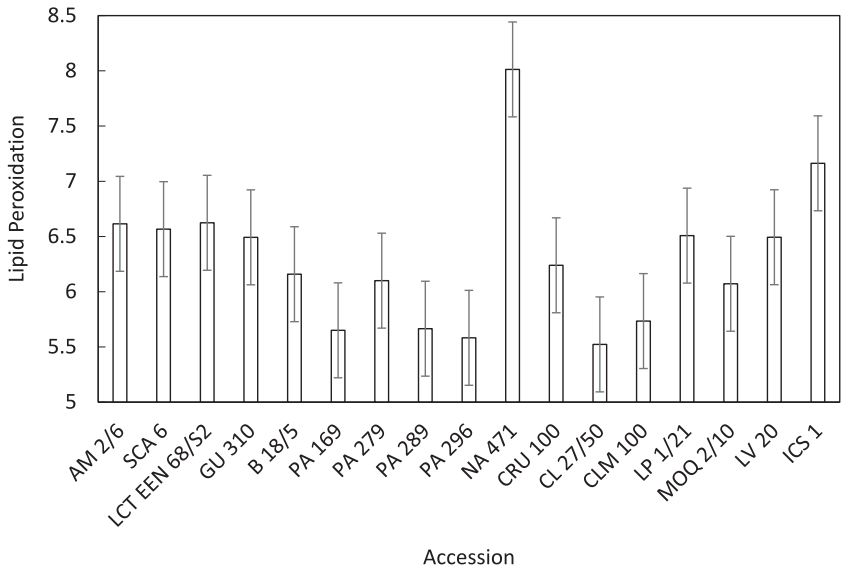

Figure 6. Genetic variation in lipid peroxidation of seventeen cacao genotypes grown under high light conditions. Mean comparisons were performed using ANOVA at $P \leq 0.05$. Bars represent mean ± SEM (n = 6).

correlations between light saturation point, ETR_{max} and quantum yield, suggesting that accessions with a higher saturation point were capable of utilising more of the light energy through photosynthetic quenching. There were however no significant correlations between the F_v/F_m values and the other photosynthetic parameters measured. This suggests that the ability to use a greater proportion of the light energy through photosynthetic quenching did not protect the plants from photoinhibition during the day (perhaps due the amount of excess light, which was substantially above the light saturation point for all accessions). If cocoa is to be grown under an orchard system, then accessions that demonstrate high light saturation points and limited photoinhibition such as AM 2/6 and B 18/5 should be chosen. As cacao plants demonstrate light saturation points below the PPFD experienced under full sun, growing cacao under high light will require accessions with high levels of protective mechanisms (for light energy dissipation and ROS scavenging).

There was significant variation in the total chlorophyll content of the accessions used in this study, suggesting that there is genetic variation in the pigment content between accessions. The range of values reported in this study is consistent with those previously reported by Suárez Salazar (2018) that, depending on the light conditions, fell within the range of 1.3–2.04 mg g^{-1} DW and Lennon et al. (2021) who reported a value of 3.94 mg g^{-1} DW for sun leaves. Variation between studies may reflect either genetic variation between the accessions used in the studies or different light conditions that prevailed under the study conditions. Likewise, similar variation was seen in total carotenoid content and the range in this study which is consistent with that of Suárez Salazar (2018) and Lennon et al. (2021). The absolute values of photosynthetic pigments determined in this study were at the low end of that seen in tropical trees (Anser and Martin, 2008, 2016) and approximately 50% of that of sun leaves of trees from temperate regions (Lichtenthaler and Buschmann, 2001; Lichtenthaler et al., 2007).

The chlorophyll *a/b* ratio for sun exposed leaves usually lies between 3.0-3.8 and the ratio for shade leaves between 2.4-2.7 (Lichtenthaler and Buschmann, 2001). In this study the accessions, apart from LV 20, demonstrated *a/b* ratios consistent with sun leaves suggesting that although cocoa is typically a shade plant there is pigment plasticity to respond to high light. The *a/b* ratio is a measure of the photosystems and light harvesting complexes content and the light adaptation of the photosynthetic equipment (Lichtenthaler et al., 1981). Chlorophyll *a* is

found in the reaction centres and the antenna systems of both photosystems whereas chlorophyll *b* is not present within the reaction centres with the majority being found in the light harvesting complex II (LHC-II).) The a/*b* ratio of the light harvesting complex I (LHC-I) has a value of 3 whereas that of LHC-II is in the range of 1.1–1.3 due to its higher content of chlorophyll *b*. In general shade plants display a lower *a/b* ratio compared to sun plants as they contain more LHC-II due to a larger antenna system (Lichtenthaler et al., 1982, 1984). LV 20 displayed the *a/b* ratio characteristic of shade leaves even when grown under high light. This suggests that not all cocoa accessions are able to adjust their photosynthetic apparatus in response to high light.

The total chlorophyll to carotenoid ratio of sun exposed leaves usually lies within the range of 4.2–5 (Lichtenthaler and Buschmann, 2001). In this study the accessions AM 2/6, B 18/5, PA 289, CL 27/50, ICS 1 and LV 20 had a ratio higher than 4.2. The other accession in this study had ratios below 4.2 with PA 169 showing the lowest value of 3.6. The lower values obtained in this study possibly represents light induced damage to the photosynthetic equipment with the lower value obtained due to a greater rate of breakdown of chlorophylls compared to that of carotenoids (Lichtenthaler and Buschmann, 2001). The lower values obtained in these accessions may also be due to the plants inability to manufacture additional carotenoids as a response to elevated light conditions. Values reported by Lennon et al. (2021) and Suárez Salazar (2018) were also lower than 4.2 but these studies recorded higher PPFD than that recorded in the current study. It is possible that under full sunlight those accessions in this study with ratios above 4.2 would also display reduced values in response to increased PPFD. In general, it does appear that there is genetic variation in the chlorophyll to carotenoid ratio in cacao. An ideal cocoa accession growing at high light intensities would have a high total chlorophyll and high total carotenoid content. The accessions that appear to be more suited to grow at high light are CRU 100 (2.999 ± 0.273; 0.909 ± 0.053 mg g^{-1} DW) and B 18/5 (3.692 ± 0.273; 0.847 ± 0.053 mg g^{-1} DW) (Table 2). These same accessions also demonstrated a greater ability to utilize higher light energy.

The enzymatic mechanisms protecting the cells from ROS toxic effects include SOD, APX and CAT. SOD scavenges superoxide radicals and forms hydrogen peroxide and oxygen with APX and CAT converting the hydrogen peroxide into water and oxygen (Dumanovic et al., 2021). From this study, variation in SOD, APX and CAT activity were observed between the various accessions under moderately high light condition (Figure 4). SOD values varied between 1287 ± 96 to 1982 ± 96 U g^{-1} DW, and a previously reported value of 1382 ± 98 U g^{-1} DW for sun leaves of the WAA accession (Lennon et al., 2021) falls within the range seen during this study. Lennon et al. (2021) observed no plasticity in SOD activity between the sun and shade leaves, but it does appear from the current study that there is variation in SOD activity between accessions. Lennon et al. (2021) did report plasticity between shade and sun leaves for APX activity and this study shows greater variation in APX activity between accessions than for SOD activity. The value for APX activity reported by Lennon et al. (2021) falls within the range of values for the current study. Similarly, for CAT activity the values reported for sun and shade leaves of the WAA accession demonstrated plasticity and fall within the range of values determined for the 17 accessions used in the current study. In general, the levels of SOD, APX and CAT activity are low compared to other shade tolerant tree species such as coffee (*Coffea Arabica*) (Matos et al., 2009). Comparisons between this study and that of Matos et al. (2009) demonstrate that under the sunlit canopy position, the activity of all three enzymes were significantly higher in the coffee trees. SOD, APX and CAT activity was 61–72 %, 27–80 % and 66–80 % higher in coffee trees, respectively. From this study, the activity of the three enzymes fit into the ranges seen under the heavily shaded canopy position of the coffee trees. This suggests that leaves of cocoa are less adapted to growth in high light conditions compared to other shade tolerant species. There was a significant strong positive correlation between APX and CAT suggesting a coordinated response to ROS generated by these two enzymes. However, there was no correlation between SOD and CAT and a significant moderate negative correlation between SOD and APX suggesting that the activity of SOD in cacao is regulated independently of the other two enzymes.

Significant variation in electrolyte leakage and lipid peroxidation were observed between the 17 accessions studied, however no correlation was observed between the two parameters suggesting that the electrolyte leakage observed was not due to membrane damage. A similar result was observed in the WAA accession in the study of Lennon et al. (2021). Electrolyte leakage accompanies plant response to stresses with most of the leakage a result of the opening of K^+ channels in the plasma membrane in response to the increased presence of ROS (Demidchik et al., 2014; Demidchik, 2018). When the plant experiences a high degree of stress it can result in programmed cell death whilst moderate stress can result in stress acclimation and metabolic adjustment (Demidchik et al., 2014; Dietz et al., 2016). Experiments carried out by Miyaji et al. (1997a, 1997b) showed that canopy leaves exposed to high light displayed a reduction in their life span and increased respiration rates when compared to shade leaves. Lennon et al. (2021) suggested that this reduced life span was due to higher levels of electrolyte leakage leading to metabolic adjustment. They also suggested that increased respiratory rates seen by Miyaji et al. (1997a, 1997b) were required to provide energy for catabolic process such as repair of cellular damage and acclimation to higher light intensities. In this current study, SOD demonstrates a significant and very strong negative correlation with electrolyte leakage suggesting that those accessions with higher SOD activity and low electrolyte leakage may be better suited to high light conditions and require less energy to be expended on repair and acclimation to the light stress. APX activity demonstrated a strong positive correlation with electrolyte leakage suggesting that the leaves were able to respond to the stress induced by the high light by increasing APX activity, however it is the levels of SOD that are limiting the response and the consequent electrolyte leakage.

The current study shows significant genetic variation between the various cocoa accessions for the parameters measured. Overall, the accessions from this study that are most capable of performing under moderately high light appear to be AM 2/6, B 18/5 and CRU 100. These accessions had relatively high photosynthetic pigments and parameters, high enzyme activity, moderately low lipid peroxidation and low electrolyte leakage with the exception of CRU 100 (16.65 ± 0.49%) which demonstrated high electrolyte leakage. The accessions that are most susceptible to the moderately high light are PA 169 and PA 279, as well as CL 27/50 and CLM 100. These accessions had relatively low photosynthetic pigments and parameters with the exception of PA 169 demonstrating a high saturation point, low enzyme activity, low lipid peroxidation and moderately high electrolyte leakage.

The ability of the cacao accessions to acclimatize to high light conditions was limited and the photoprotection responses appear to be restricted by the capacity to generate sufficient SOD. Nonetheless, all of the parameters tested, including SOD, showed significant variation between accessions suggesting that cacao has considerable genetic variation in tolerance to high light. Overall, cocoa accessions with high SOD activity, high carotenoid levels and high light saturation points appear to be better adapted for high light conditions. The study suggests that SOD activity, carotenoid content and light saturation point are potentially good markers to use for screening cocoa accessions for high light tolerance.

Although genetic variation was observed between the accessions studied, further studies are required to examine if particular genetic groups show enhanced capabilities for high light growth. Alongside the parameters measured in this study other factors will play a role in the adaptation of cocoa plants to high light intensities. Variation is leaf area index and canopy structure will also affect plant adaptation to growth under high irradiance. Further studies are therefore needed if cocoa breeding programmes are to produce plants capable of acclimating to an orchard growth system.

Declarations

Author contribution statement

Vernessa R. Lewis: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Aidan D. Farrell: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Pathmanathan Umaharan: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Adrian M. Lennon: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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