

SCIENTIFIC REPORTS



OPEN

Acclimation responses of macaw palm seedlings to contrasting light environments

Adriel N. Dias¹, Advanio I. Siqueira-Silva^{2,4}, João P. Souza², Kacilda N. Kuki³ & Eduardo G. Pereira²

The photosynthetic adjustments of macaw palm (*Acrocomia aculeata*) were evaluated in 30-day-old seedlings exposed to high and low light environments, and sudden transference from low to high light and comparisons were made with the hardening protocol used in nurseries. Furthermore, we evaluated the responses to long-term exposure (265 days) to high and low light environments. Macaw palm seedlings exhibited an efficient mechanism that maximized light capture under scarce conditions, and dissipated excess energy to avoid damaging to the photosystem II under high light. The seedlings showed low saturation irradiance but no photoinhibition when exposed to excess light. When grown under low light intensities, seedlings presented higher photochemical efficiency and minimized the respiratory costs with positive carbon balance at lower irradiance than hardened seedlings did. The hardening procedure did not appear to be an advantageous method during seedling production. Long-term exposure to either low or high light did not cause significant leaf anatomical adjustments. However, the low light seedlings showed higher leaf area and chlorophyll content than those exposed to higher light intensity did, which enabled shaded seedlings to maximize the captured light. Furthermore, the high non-photochemical dissipation allowed rapid acclimation to excessive light exposure. These responses allow macaw palm cultivation and establishment in very different light environments.

Light is an essential resource for the growth and development of plants, and the main determining factors are intensity, quality, duration, and distribution of the photoperiod. However, light is also a limiting resource to the growth and reproduction of some palm species¹. To cope with shaded environments, plant species generally have two adaptive strategies, namely shade avoidance and tolerance². Those that avoid shade (shade intolerant, light demanding) use the resources available in the environment to grow toward the light, as opposed to shade-tolerant species that preferentially exhibit efficient absorption and use of the scanty light energy under shade conditions. Acclimation to environments with low light intensities may involve changes in the chloroplast, leaf and plant levels³. Generally, neotropical palm species are shade tolerant, and they show some important leaf responses such as low compensation irradiance, epidermal chloroplasts, and horizontally oriented leaves with a long lifespan⁴.

The leaf, the main vegetative plant organ for capturing light, is considered highly plastic and, consequently, it is most evaluated in studies of the effects of abiotic stresses on plants⁵. The ability of plants to acclimate to different irradiances is a function of the morphophysiological adjustments of their leaves. These responses may vary among plant species or even with different development stages of a single species⁶. The amount of light that reaches leaves during development can cause structural adjustments in these organs, with significant changes in the thicknesses of the mesophyll and leaf blade, stomatal density, and the volume of the intercellular spaces^{7,8}. There may also be changes in the area and mass of leaves^{9,10}, and the efficiency of light interception depending on leaf orientation, shape, and size¹¹.

In addition to the morphoanatomical plasticity, some plant species can adjust their photosynthetic responses to changes in irradiance, and these physiological variables play a decisive role in the acclimation of the plant to a

¹Programa de Pós-graduação em Manejo e Conservação de Ecossistemas Naturais e Agrários, Universidade Federal de Viçosa (UFV), Campus Florestal, Florestal, Minas Gerais, Brazil. ²Instituto de Ciências Biológicas e da Saúde, UFV, Campus Florestal, Florestal, Minas Gerais, Brazil. ³Departamento de Fitotecnia, UFV, Campus Viçosa, Viçosa, Minas Gerais, Brazil. ⁴Present address: Universidade Federal do Oeste do Pará (UFOPA), Campus Universitário de Juruti, Juruti, Pará, Brazil. Correspondence and requests for materials should be addressed to E.G.P. (email: egpereira@ufv.br)

shaded or full-sunlit environment^{6,7,9}. The ability of plants to allocate biomass and survive under the most diverse lighting conditions depends on their photosynthetic capacity¹¹, preventing photoinhibition under exposure to excessive light, or maximizing light capture when this resource is scarce¹². A sudden increase in irradiation in the understory due to the opening of clearings in the forest canopy can result in physiological stress and determine the distribution pattern of some palm species⁹. A similar seasonal effect can also be observed in semi-deciduous tropical forests when the leaves fall from the canopy, which increases the light intensity that reaches the lower stratum¹³. This increased light intensity is a disadvantage to shade-tolerant species, whereas it favors the pioneers or the light-demanding species¹³. Although palms are a regular fixture of the tropical vegetation, most information about their carbon physiology is limited to crop species including *Eleais guineensis* and *Cocos nucifera*.

The oleaginous palm *Acrocomia aculeata* (Jacq.) Lood. Ex Mart. (Arecaceae), also known as macaw palm, is found in savanna-like vegetation, semi-deciduous seasonal forests, and deforested areas, among other physiognomies of Central and South America^{14,15}. It preferentially occurs in areas of higher natural soil fertility but also can occur in sandy and low-fertile soils¹⁶. Because of its high productivity and oil traits, the palm has great potential for the industrial sectors in the production of vegetable oil, for pharmaceutical, cosmetics, food, and biofuel development¹⁷. Native to the neotropics, the macaw palm is classified as a pioneer species¹⁸, and it has remarkably drought tolerance¹⁹. Despite being described as a heliophytic species, little is known about the acclimatory responses of young macaw palm plants to light.

The standard protocol to overcome dormancy and promote germination of macaw palm seeds²⁰ was established by Motoike, *et al.*²¹ (Patent registration: PI0703180-7 A2). The recommended initial seedling cultivation process consists of using pre-germinated seeds in two consecutive stages: the pre-nursery stage, which extends from pre-germination to the appearance of the first pair of lanceolate (split) leaves and the nursery stage that extends to the appearance of the second pair of pinnate (mature) leaves. Starting from this point when the seedlings are approximately 1 year old, field planting can begin²². Generally, after the formation of the first eophyll in the pre-nursery stage, a hardening procedure is empirically used to promote the robustness of seedlings and enhance their capacity to acclimate to excessive light, thereby preventing photoinhibitory stress in the nursery and hastening development in the field²³. During the hardening procedure in macaw palm, the young seedlings are daily transferred from the shading cloth structure of the pre-nursery (low light environment) to a higher light intensity, expect in the hours when irradiance levels are at the highest, i.e., between 10:00 am–4:00 pm. However, currently, no studies have evaluated the morphological and physiological responses of young macaw palm plants to contrasting light environments.

Field-grown macaw palm plants exhibit high photosynthetic efficiency under high irradiances and can maintain carbon assimilation by low compensation and saturation irradiance¹⁸. Therefore, we investigated the hypothesis that young *A. aculeata* plants have high acclimation capacity and low susceptibility to photoinhibition when exposed to contrasting irradiance conditions. The objective of this study was to evaluate the photosynthetic and morphoanatomical adjustments of the macaw palm seedlings to different light intensities.

Methods

Plant material and experimental conditions. The study was conducted at the Federal University of Viçosa, *Campus Florestal* (19°52'S, 44°25'W). The seeds of *A. aculeata* were collected in the central region of the State of Minas Gerais, Brazil. The protocol for overcoming dormancy and the germination process was performed according to the method of Motoike, *et al.*²¹. The seedlings were transferred to tubes (180 cm³) containing a commercial substrate Tropstrato[®] HT containing 1 kg m⁻³ of single superphosphate fertilizer. During the first 30 days, the seedlings were grown under low light environment (maximum irradiance of 320 μmol m⁻² s⁻¹) until the emergence of the first eophyll. Subsequently, two assays with different durations of the treatment exposure were conducted.

In the first assay, during the pre-nursery stage, 1-month-old macaw palm seedlings were exposed for 30 days to the following three treatments. (i) The seedlings were grown in a greenhouse under high light conditions throughout the entire period (maximum irradiance of 1030 μmol m⁻² s⁻¹). (ii) The seedlings remained shaded (low light condition) under a 2.10 m high × 1.80 m wide structure, covered with a shade cloth typically used in nurseries (maximum irradiance of 320 μmol m⁻² s⁻¹). (iii) The seedlings were subjected to the hardening procedure and were maintained under low light during the day with the highest irradiance (10:00 am–4:00 pm), and then they were transferred to high light conditions prior to or after this period. In addition, after the first 30 days, some of the seedlings that were under the low light treatment were exposed to a new treatment: (iv) the seedlings were directly transferred to high light condition, without the process of hardening. Immediately after this treatment commenced, physiological and morphological evaluations of seedlings from all treatments were performed on the second split-leaves with typical parallel veins²⁴, which developed after the eophyll under each lighting condition.

In the second assay, at the nursery stage, young macaw palm plants (3-month-old) from the high light exposure and low light treatments were transplanted into polyethylene bags (5 L) filled with substrate, containing a soil mixture:sand:cattle manure (2:1:1), enriched with 1 kg m⁻³ dolomitic limestone, 6 kg m⁻³ single superphosphate, 1 kg m⁻³ ammonium sulfate, and 0.30 kg m⁻³ potassium chloride. The trial consisted of long-term exposure (over 265 days) of the seedlings to the two contrasting light treatments in the same setting as the first assay. Throughout the exposure period, the chlorophyll *a* fluorescence, photosynthetic pigment content, specific leaf area (SLA), growth variables, and leaf anatomy were evaluated.

Evaluation of photosynthetic responses of macaw palm seedlings under contrasting irradiances. In the first assay (short-term exposure to contrasting light conditions), we evaluated the net photosynthetic rate (*A*, μmol m⁻² s⁻¹), stomatal conductance (*g_s*, mol m⁻² s⁻¹), transpiration rate (*E*, mmol m⁻² s⁻¹), the ratio between internal and external CO₂ concentration (*C_i/C_a*), and instantaneous water use efficiency

($W_i = A/E$, $\mu\text{mol}_{\text{CO}_2} \text{mmol}_{\text{H}_2\text{O}}^{-1} \text{m}^{-2} \text{s}^{-1}$) in seedlings in all treatment groups for 9 days, from the day the seedlings were transferred to high light conditions. Gas exchange measurements were performed between 08:00 am and 12:00 pm using an infrared (IR) gas analyzer (model LI-6400XT, Li-Cor Inc., Lincoln, NE, USA) at light intensity of $1,000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ provided by a light source (6400-40 Leaf Chamber, Li-Cor Inc., Lincoln, NE, USA) for all plants. The CO_2 concentration was set to $400 \mu\text{mol mol}^{-1}$ using a CO_2 injector system (6400-01, Li-Cor Inc., Lincoln, NE, USA). Other climatic parameters were not controlled during the assay (mean ambient temperature of $27 \pm 3^\circ\text{C}$ and mean vapor pressure deficit of $2.2 \pm 0.3 \text{ kPa}$).

The chlorophyll *a* fluorescence variables F_0 and F_v/F_m were measured using the Mini-PAM modulated pulse fluorometer (Heinz Walz, Effeltrich, Germany) in the same leaflet region where the gas exchange was measured in plants in the first experiment. Briefly, the leaflets were acclimatized in the dark for 30 min and then exposed to a weak pulse of red light ($0.15 \mu\text{mol m}^{-2} \text{s}^{-1}$) to determine the F_0 . Then, a pulse of saturating light at $12,000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ was applied for 0.8 s, to determine the maximum fluorescence (F_m). The obtained data were used to estimate the $F_v/F_m = (F_m - F_0)/F_m$ ²⁵. The chlorophyll content index was evaluated in the first assay using the portable chlorophyll meter ClorofiLOG (Falker, Porto Alegre, RS, Brazil) before the fluorescence analysis and three measurements were performed on each leaf blade.

In the second assay (long-term exposure to constant high light and low light), foliar pigments were quantified using dimethyl sulfoxide (DMSO) as the extractor. Briefly, at the end of the trial, leaf discs with an average dry mass of 7.5 mg, were removed from each replicate evaluated and incubated in 5 mL saturated DMSO in calcium carbonate (CaCO_3). To speed up the pigment extraction, the samples were kept in water bath at 65°C for 36 h, and the absorbance was read at 480, 649.1, and 665.1 nm. The photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids) were determined according to the method of Wellburn²⁶. The results were expressed in $\text{mg g}_{\text{DW}}^{-1}$.

Evaluation of photosynthetic light response curves in macaw palm seedlings. Light response curves of the net *A* and effective quantum yield of photosystem II (ϕ_{PSII}) were constructed for seedlings from the first experiment on day 14 after the plants were transferred to full sunlight conditions, using the autoprogram function of the LI-6400XT IR gas analyzer attached to a fluorescence chamber. Briefly, the leaflets were exposed to decreasing actinic light intensities (2000, 1500, 1000, 500, 200, 100, 50, 20, and $0 \mu\text{mol m}^{-2} \text{s}^{-1}$) for a minimum and maximum time of 120 and 200 s, respectively for each light intensity, and a CO_2 concentration of $400 \mu\text{mol mol}^{-1}$ under the same conditions described previously. The light response curves of the net *A* obtained were adjusted using the model that best fit the data, the hyperbolic rectangular model²⁷, using *Microsoft Excel 2013*²⁸. We evaluated the dark respiration (R_D), light compensation point (I_{comp}), maximum net *A* (A_{max}), the light saturation point beyond which there is no significant change in net photosynthesis (I_{max}), and the maximum quantum yield (ϕ , taking the linear response of *A* between I_{comp} and $200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). The light response curves of the ϕ_{PSII} were adjusted using exponential decay and linear equations where appropriate.

In the second assay, rapid light response curves were obtained by using the Mini-PAM fluorometer to determine the instantaneous capacity of photosynthetic response to excessive solar radiation in macaw palm seedlings. At first, the leaflets were dark-acclimated for 30 min to determine F_0 and F_m . Then, the leaflets were exposed to the light conditions where plants grown for at least 10 min and the light response curve was started. The leaflets were exposed to increasing actinic light intensities (0 – $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$) during nine 10-s intervals, and the steady-state fluorescence (F_s) and maximum light-adapted fluorescence (F'_m) were measured. Based on these values, we calculated the ϕ_{PSII} ²⁵ and the quantum yield of regulated (ϕ_{NPQ}) and non-regulated (ϕ_{NO}) energy dissipation²⁹. The photochemical quenching (q_L)³⁰ and non-photochemical quenching (q_N)³¹ were also calculated. The values for minimum fluorescence in the light-adapted state (F'_0) were obtained according to Oxborough and Baker³². The apparent electron transport rate (*ETR*) was calculated as: $ETR = \phi_{\text{II}} \times \text{PAR} \times I_A \times 0.5$; where PAR = photosynthetically active radiation, I_A = the leaflet absorptivity coefficient, and 0.5 = fraction of excitation energy distribution in PSII³³.

Evaluation of photoinhibition and contribution of non-photochemical components to de-excitation of PSII in macaw palm seedlings. The extension of photoinhibition and recovery capacity were evaluated in macaw palm seedlings in the first assay on day 20 after the commencement of the direct transfer treatment following the protocol of Zivcak, *et al.*³⁴ with modifications. In pre-dawn, the F_v/F_m measurements were determined using the Mini-PAM fluorometer. Then, the macaw palm leaves were exposed to $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 5 min, and soon after that, a light pulse was administered to determine the ϕ_{PSII} . Then, the leaves were exposed to the photoinhibitory condition with light intensity of $3000 \mu\text{mol m}^{-2} \text{s}^{-1}$, (from an external light source 2050-HB Heinz Walz, Effeltrich, Germany). A filter eliminating wavelengths above 710 nm and a ventilator were used to exclude IR radiation and over-heating of the leaf. Light saturating pulses ($12,000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) were applied at each 10-min interval over a 40-min period. Subsequently, the plants were allowed to recover from the photoinhibitory treatment in the dark, with photochemical efficiency measurements at 1, 15, and 30 min. Then, 24 h after the high light treatment, a new F_v/F_m evaluation was performed at the pre-dawn stage to characterize the extent of photoinhibition.

The contribution to the different components of q_N to the de-excitation mechanisms was determined using dark relaxation kinetics of fluorescence in plants from the second experiment. First, the F_0 and F_m were determined at dawn. Then, in the morning (08:00 am) the leaflets were exposed for 2.5 min to $3000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. After 1, 5, 20, and 90 min exposure to excess irradiance, the F_0 and F_m were measured³⁵. The three components of q_N , energy-dependent quenching (q_E), state transition quenching (q_T), and photoinhibitory quenching (q_I), have different relaxation times. The fastest, relaxation time (2–5 min.) indicates the dissipation through q_E , the intermediate relaxation time (12–20 min), indicates the dissipation by q_T ; and the slow relaxation time (>40 min) indicates the degree of dissipation by the photoinhibition mechanism³⁵.

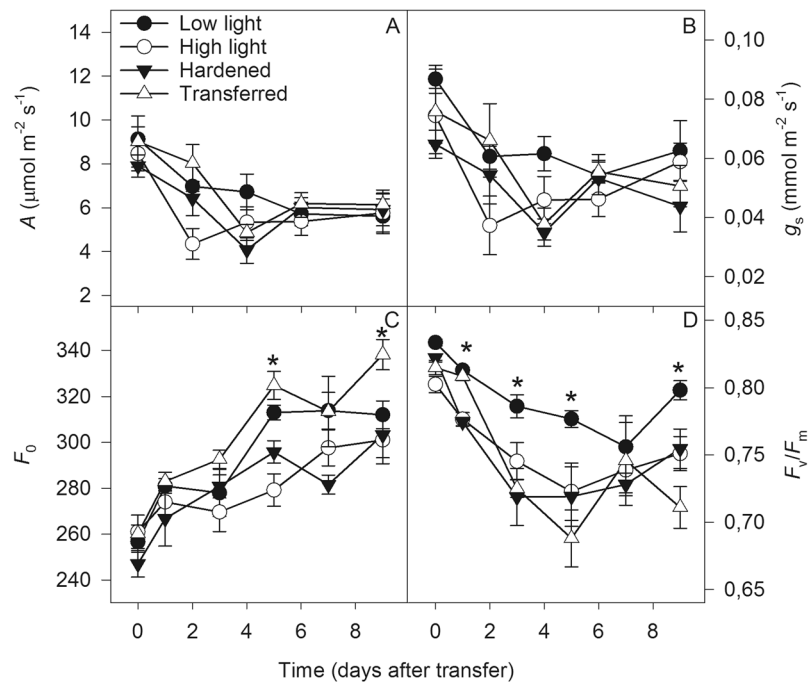


Figure 1. Photosynthesis (A – **A**) stomatal conductance (g_s – **B**) initial fluorescence (F_0 – **C**) and maximum quantum efficiency of photosystem II (PSII; F_v/F_m – **D**) in macaw palm seedlings grown in the short term under pre-nursery stage of contrasting light conditions. The bars indicate the standard error of the mean of five replicates and the asterisk indicates significant difference by the Tukey test, at 5% probability.

Morphoanatomy traits and leaf micromorphometry of macaw palm seedlings grown under contrasting light conditions. In the first assay, the leaf area was determined on day 27 after the direct transfer treatment started and periodically throughout the long-term shading in the second experiment, using a portable leaf area meter (LI 3000C, Li-Cor Inc., USA). In the second assay, the diameter and length of the stipe were also evaluated using an analog caliper and millimeter ruler, respectively.

To evaluate the SLA, a leaflet was collected from each plant in the second assay. The leaf area (LA) was determined as shown previously. The leaflets were then dried at 75 °C until a constant mass was obtained (48 h) for the dry mass (DM) quantification. The SLA was calculated as the ratio of the LA to DM.

For the light microscopy structural analyses, samples from the middle region of fully expanded leaflets grown under long-term low and high light conditions (second experiment) were fixed in formaldehyde-acetic-alcohol (FAA) 70 solution (37% formaldehyde, 100% glacial acetic acid, and 70% ethanol, 1:1:18, v/v/v)³⁶ for 48 h. They were then dehydrated in an ethanol series and embedded in Leica histo-resin (Leica Microsystems Inc., Heidelberg, Germany), according to the manufacturer's recommendations. Cross sections (5 μm) were cut using a rotary microtome, stained with 0.05% toluidine blue in 0.1 M phosphate buffer pH 6.8³⁷, and mounted in Entellan[®]. The images were acquired using a photomicroscope (model BX41 TF, Olympus Optical, Tokyo, Japan) equipped with an image capture camera (model SC 30, Olympus Soft Imaging Solutions GmbH, Munster, Germany).

For the micromorphometric analyses, three leaflets were collected from each plant ($n = 5$), fixed in FAA 70 solution, and one sample from each replicate was randomly processed, following the methodology of the structural characterization. The height of the epidermal cells, the hypodermis of both faces of the leaflet, and the thickness of the mesophyll and the limbus were measured. The six parameters were measured three times per histological section, in five photomicrographs of each replicate ($n = 5$) of the shading and full sunlight conditions using the AxioVision 4.9.1 software, Carl Zeiss Microimaging GmbH, Jena, Deutschland.

Statistical analysis. A randomized block design ($n = 5$) was used for all the experiments, and each experimental unit was composed of a macaw palm seedling. The data were analyzed using an analysis of variance (ANOVA), where the means were previously evaluated using the Breusch-Pagan homogeneity of variance test and the Shapiro-Wilk variance homogeneity test (5% probability) using the statistical program R version R i386 3.1.2. Comparisons between means were performed using the Tukey test, at a 5% probability.

Results

Variation in short- and long-term light intensity did not change net A of macaw palm seedlings. No significant changes were observed in the A and g_s (Fig. 1A,B) or E , C_i/C_a and W_t (Supplementary Fig. S1) of the macaw palm seedlings in response to the different lighting conditions or the sudden variation in light intensity under pre-nursery conditions. However, plants transferred directly from low to high light showed increased F_0 with significant differences ($p < 0.05$) on the day 5 of evaluation when compared with the hardened and high light plants, and on day 9 with plants from the other treatments (Fig. 1C). In general, the low light

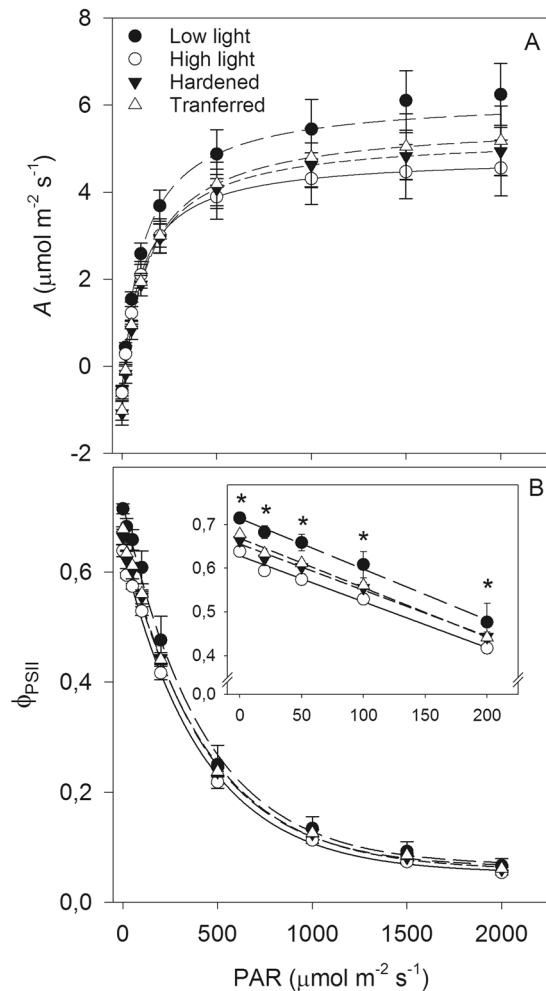


Figure 2. Light-response curves of net photosynthetic rate (A – **A**) and effective quantum yield of photosystem II (PSII; ϕ_{PSII} – **B**) in macaw palm seedlings grown in the short term under pre-nursery stage of contrasting light conditions. The inner panel indicates the magnified area from 0 to 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The bars indicate the standard error of the mean of five replicates and the asterisk indicates significant difference by the Tukey test, at 5% probability.

seedlings showed significantly ($p < 0.05$) higher F_v/F_m values compared to the other treatments (Fig. 1D). During the evaluation period, was observed a decrease in A , g_s and F_v/F_m , as a consequence of increasing F_0 , independent of the applied treatments (Fig. 1).

The seedlings grown under high light conditions showed significantly ($p < 0.05$) lower chlorophyll content indexes than seedlings that were transferred after 2 and 4 days did. No differences were found over time or among the other treatments (Supplementary Fig. S2). After 265-day long-term exposure to low and high light treatments, macaw palm plants showed significant ($p < 0.05$) difference in the levels of chlorophylls (a , b , and total) and carotenoid, with higher values found in low light plants. No significant changes were detected in the ratios of chlorophyll a/b and carotenoids/chlorophylls between treatments (Supplementary Fig. S3).

Macaw palm presented high photochemical efficiency at low irradiance and effective energy dissipation under excessive light conditions. Plants from all the contrasting light conditions administered in the pre-nursery stage showed similar patterns in light response curves of the net A (Fig. 2A). Young macaw palm seedlings exhibited a mean I_{max} of 105 $\mu\text{mol m}^{-2} \text{s}^{-1}$, high quantum efficiency in carbon fixation, and maximum net A of 5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ on average. No significant differences ($p > 0.05$) between treatments were observed for these variables (Table 1). However, plants that were directly transferred to high light and those subjected to the hardening procedure had a significantly higher dark respiratory rate (R_D) than plants from other treatments did (Table 1). The plants maintained in the lower irradiance presented the lowest I_{comp} values, differing significantly from those of the hardened plants, which showed the highest values (Table 1).

At low irradiance ($< 500 \mu\text{mol m}^{-2} \text{s}^{-1}$) the differences in the light response curve of the ϕ_{PSII} between the treatments were highlighted, with higher ϕ_{PSII} values shown by low light seedlings than by those exposed to high light treatment (Fig. 2B).

In addition to the non-significant ϕ_{PSII} values between treatments (Fig. 3A), the long-term exposure (265 days) of seedlings to low and high light conditions changed the light response curve of some chlorophyll a fluorescence

Treatments	R_D	I_{comp}	A_{max}	I_{max}	$\Phi_{(I_{comp} - I_{200})}$
Shaded	0.6 ± 0.1 b	9.6 ± 1.5 b	5.8 ± 0.8 a	113.2 ± 18.8 a	0.017 ± 0.002 a
Full Sunlight	0.6 ± 0.1 b	13.4 ± 3.7 ba	4.5 ± 0.6 a	88.8 ± 12.7 a	0.014 ± 0.002 a
Hardened	1.1 ± 0.3 a	23.8 ± 5.9 a	4.9 ± 0.5 a	107.4 ± 11.1 a	0.015 ± 0.001 a
Transferred	1.0 ± 0.2 a	20.3 ± 1.7 ba	5.2 ± 0.8 a	110.2 ± 12.2 a	0.015 ± 0.001 a

Table 1. Light response curve variables from macaw palm seedlings grown in the short term under pre-nursery stage of contrasting light conditions. R_D – dark respiration [$\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$]; I_{comp} – light compensation point [$\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$]; A_{max} – maximum photosynthetic rate [$\mu\text{mol} \text{m}^{-2} \text{s}^{-1}$]; I_{max} – light saturation point beyond which there is no significant change in net photosynthesis [$\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$]; $\Phi_{(I_{comp} - I_{200})}$ – maximum quantum yield at the range between I_{comp} and $I = 200 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ [$\mu\text{mol}(\text{CO}_2) \mu\text{mol}(\text{photon})^{-1}$]. Means ± standard error followed by the same letter in a column do not differ from each other according to Tukey's test at 5% significance level. (n = 5).

variables. Seedlings exposed to high light presented significantly ($p < 0.05$) higher ETR values than low light seedlings did (Fig. 3B), irrespective of irradiance level. In contrast, low light seedlings showed significantly higher ($p < 0.05$) energy dissipation through q_N mechanisms (Fig. 3C) and related to the higher Φ_{NPQ} (see Supplementary Fig. S4). The q_L and Φ_{NO} did not change significantly in response to the treatments (Supplementary Fig. S4).

Effective photoinhibition recovery and photoprotection through dissipation of excess energy occurred in macaw palm seedlings.

The macaw palm seedlings cultivated under contrasting light environments showed similar responses to the photosynthetic quantum yield during the initial exposure (5 min) to low light intensity ($50 \mu\text{mol} \text{m}^{-2} \text{s}^{-1}$) and throughout the photoinhibitory process (40 min exposure to $3000 \mu\text{mol} \text{m}^{-2} \text{s}^{-1}$). During the dark-recovery period (46–75 min), values of the Φ_{PSII} remained below the initial measurement of this variable (time zero), and the maximum recovery rate was achieved just after 1 min dark exposure. After the 24-h recovery period, the maximum photochemical efficiency values were re-attained, regardless of the treatments. Moreover, the seedlings from the low light treatment presented significantly higher ($p < 0.05$) F_v/F_m values than seedlings from the other treatments did (Fig. 4).

The exposure of young macaw palm plants to long-term low and high light treatments did not significantly change ($p > 0.05$) q_N (Fig. 5A). However, plants grown under high light presented a higher proportion of q_E than low light plants did (Fig. 5B). The other coefficients of non-photochemical quenching (q_T and q_I) did not change in response to the treatments (Fig. 5B).

Macaw palm presents limited morphoanatomic adjustments in response to light availability.

The macaw palm seedlings grown under low light conditions during the pre-nursery stage presented significantly larger ($p < 0.05$) LA than seedlings from the other treatments did, which did not differ among treatments (Fig. 6A). No significant differences ($p > 0.05$) were observed in the SLA after exposing young macaw palm plants to low and high light treatments for 265 days (Fig. 6B). However, periodical measurements of LA during the long-term exposure to different contrasting light conditions (Supplementary Fig. S5) showed a change in the LA pattern with shaded plants exhibiting higher values in the first 5 months than unshaded plants did. In addition, no significant differences between treatments were found during the remaining period ($p > 0.05$). Although the stipe increased during the development of the plantlets in the first year of life, the diameter and length of the stipe of the young macaw palm plants showed no significant difference between treatments until the end of the experiment when a greater stipe length was found in shaded plants than in those exposed to sunlight (Supplementary Fig. S5).

Macaw palms display amphi-hypostomatic leaflets (stomata are present on both sides but with predominance in the abaxial surface), uniseriate epidermis and hypodermis on both sides, compact parenchyma with more elongated and narrow cells in the adaxial surface forming the palisade mesophyll, and they contain phenolic compounds (Fig. 7A and B). The vascular bundles are collateral and covered by layers of fibers and bundle sheath. After long-term exposure (265 days) to different light conditions, no significant changes ($p > 0.05$) in leaf tissue thickness of young macaw palm plants were observed (Table 2).

Discussion

The ability of macaw palm seedlings to maximize photosynthetic responses under contrasting light conditions is due to their specialized anatomical and physiological leaf features, consistent with pioneer species⁷. Irrespective of the incident light level during the initial growth period, macaw palm seedlings exhibit efficient mechanisms for maximizing light capture when lighting is low and dissipating excess energy to avoid damaging the PSII reaction center under supra-optimum irradiance conditions. Mainly due to energy dissipation through non-photochemical quenching, macaw palm seedlings were able to maintain unaffected its net photosynthetic rate with high values of water use efficiency, regardless of the light treatment they were subjected to. These responses, optimize productivity and guarantee the establishment of the species in different environments. For photosynthetic organisms, it is essential to use the highest possible number of photons for the production of CO_2 , as well as to be able to quenching the excess energy that were not used, avoiding the transfer of this energy to reactive oxygen species, which are potentially toxic to cells³⁸. *A. aculeata* is of natural occurrence in the Cerrado bioma, where the climate is predominantly savanna-like and the vegetation arbustive and grassy. Despite the fact that gas exchange measurements can be challenging in tropical dry regions, due to intrinsic variation of climatic variables, the palm is

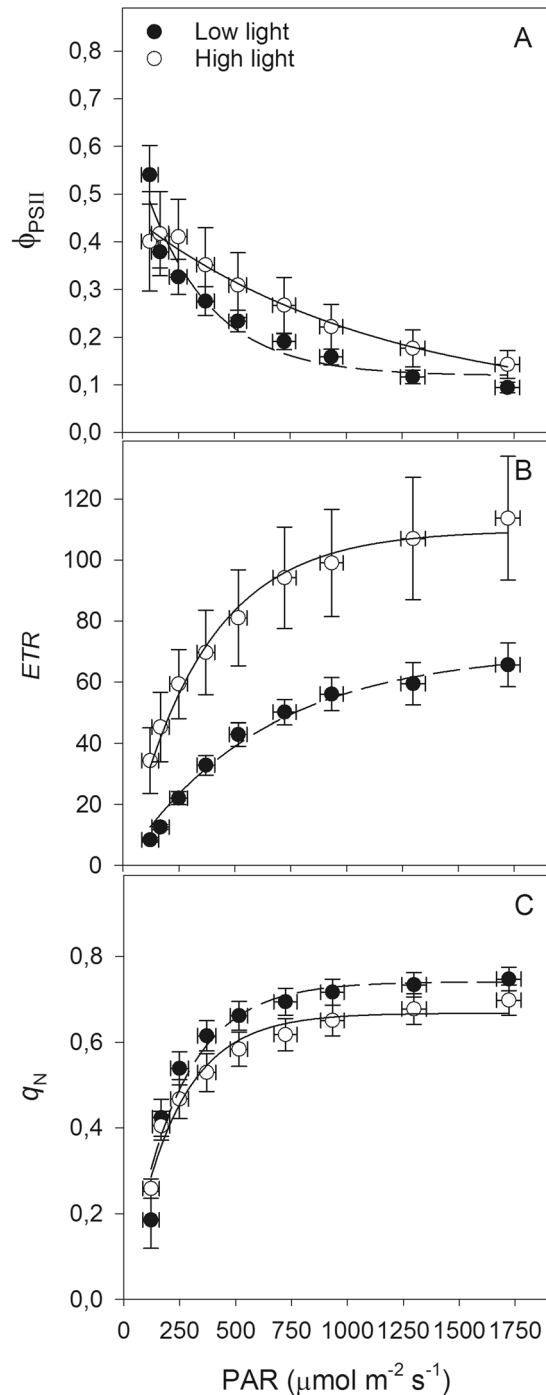


Figure 3. Light responses curves of the chlorophyll fluorescence variables: effective quantum yield of photosystem II (PSII; ϕ_{PSII} – **A**), relative electron transfer rate (ETR ($\mu\text{mol m}^{-2} \text{s}^{-1}$) – **B**) and non-photochemical quenching (q_N – **C**) in macaw palm seedlings grown in the long term under nursery conditions of low and high light. The bars indicate the standard error of the mean of five replicates. Significant differences ($p < 0.05$) were found in simple effects of treatments on the ETR and q_N variables.

well-adapted; hence this rustic species probably possesses tolerance mechanisms to secure effective CO_2 assimilation during drought or adverse climatic conditions, through effective stomata control¹⁹.

Macaw palm seedlings presented some adjustments in the efficiency of light capture highlighted by the photosynthetic light response curves. The low I_{max} ($105 \mu\text{mol m}^{-2} \text{s}^{-1}$) allows reaching the maximum potential for carbon fixation in relatively low light intensities, but without photosynthetic decay due to photoinhibition when exposed to high light intensity (up to $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$). This photoinhibitory response to excess light would be very common in some palm species originally from dense forests phytophysionomies^{9,39,40}. In addition to carbon gain, the maintenance of photosynthetic efficiency in response to contrasting light intensities

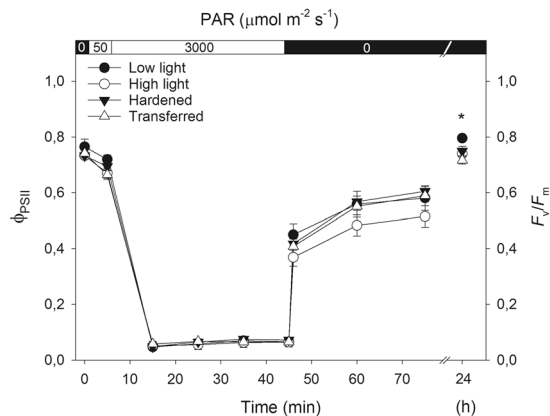


Figure 4. Photoinhibitory response of quantum yield of photosystem II in macaw palm seedlings grown short-term under pre-nursery stage of contrasting light conditions. The leaves were exposed to the following protocol: 5 min low light ($50 \mu\text{mol m}^{-2} \text{s}^{-1}$), followed by 40 min high light ($3000 \mu\text{mol m}^{-2} \text{s}^{-1}$), 30 min and 24 h of darkness. The bars indicate the standard error of the mean of five replicates and the asterisk indicates significant difference by the Tukey test, at 5% probability.

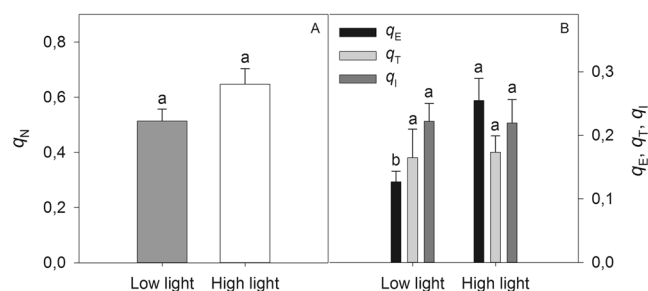


Figure 5. Non-photochemical quenching (q_N – **A**) and its three components (**B**) energy-dependent quenching (q_E); state transition quenching of PSII (q_T) and photoinhibitory quenching (q_I) in young macaw palm plants grown long-term under nursery conditions of low and high light. The bars indicate the standard error of the mean of five replicates. Different letters indicate significant difference by the Tukey test, at 5% probability.

would also be expressed as respiratory costs for maintaining a light compensation point in accordance with the availability of light in the environment⁶. Indeed, macaw palm seedlings grown under the shade minimized respiratory costs while keeping a positive carbon balance at low irradiance ($>9.6 \mu\text{mol m}^{-2} \text{s}^{-1}$). Interestingly, the high-light-exposed seedlings also showed lower R_D than those that were transferred to full sunlight or hardened did, which indicates that the maintenance cost of the photosynthetic capacity of the former treatment would be lower. In fact, in this study, the hardening procedure did not appear to be advantageous during the pre-nursery stage of macaw palm seedling production since it did not promote better acclimation responses to contrasting light environments than the other methods did.

The efficiency of light capture under low light availability and the photosynthetic response of macaw palms to high light intensities was also confirmed by the results of Pires, *et al.*¹⁸ in the light response curves of field-grown plants during the reproductive stage. Sunlight-exposed field-grown macaw palm plants showed lower compensation irradiance and higher saturation irradiance¹⁸ than young macaw-palm seedlings did. In addition, seedlings of macaw palm plants in this study presented lower ϕ values for CO_2 fixation ($0.015 \text{ mol mol}^{-1}$) than adult plants did, as reported previously¹⁸. The ϕ is a conservative variable, considering the light environment and the intrinsic traits of the leaves⁴¹ that did not exhibit significant changes among treatments. However, all these differences between seedlings and field-grown macaw palm plants indicate that the continuous adjustments of the light response curve variables are consistent with the variations in the light intensity and developmental stage⁴². While the ϕ remained unchanged in macaw palm seedlings cultivated under contrasting light environments, the higher ϕ_{PSII} of the shaded seedlings at low light intensities indicates that the light capturing and efficient use were linked to the carbon fixation at maximum photosynthetic efficiency. The values of ϕ_{PSII} obtained with rapid light-response curves were similar with those obtained by steady-state light curve at the same irradiance, which show the method's reliability and allow better comprehension of the short-term acclimation changes in photosynthetic activity of macaw palm seedlings exposed to different light availability. At higher light intensities, the similar responses of ϕ_{PSII} for increasing PAR availability in both treatments, reveal that, regardless of its light growth environment, macaw palm seedlings capture and use quantum energy in the same capacity. On the other hand, the lower ETR response of low light plants indicating that as PAR availability increased, the plants grown and acclimated to low light setting could not effectively draw the electrons through photochemical processes. In

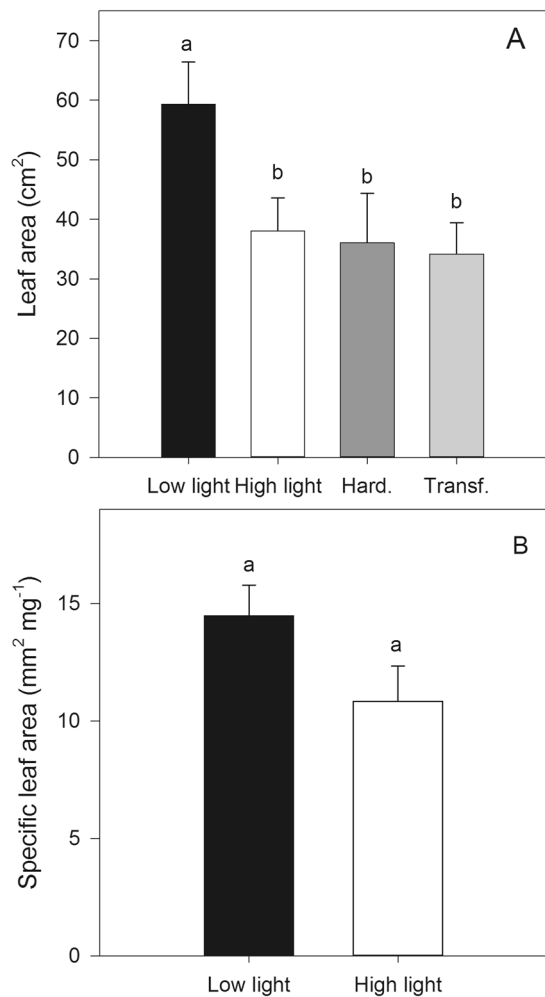


Figure 6. Leaf area (LA, **A**) of macaw palm seedlings grown in short-term under pre-nursery stage of contrasting light conditions and specific leaf area (SLA, **B**) of young macaw palm plants grown long-term under nursery conditions of low and high light. The bars indicate the standard error of the average of five replicates. Different letters indicate significant difference by the Tukey test, at 5% probability.

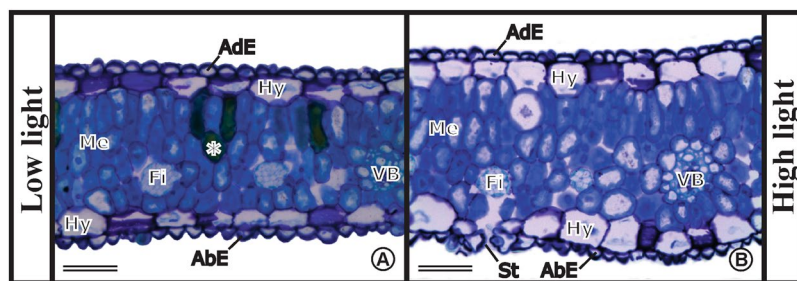


Figure 7. Cross sections of the leaflets from young macaw palm plants grown long-term under nursery conditions of low (**A**) and high light (**B**). AdE, adaxial surface epidermis; AbE, abaxial surface epidermis; Hy, hypodermis; Me, mesophyll; Fi, fiber; VB, vascular bundle; St, stoma; and (*) phenolic compounds. Scale bars = 50 μm (**A** and **B**).

such excess conditions, dissipation by heat is the ordinary mechanism to alleviate stress³⁸ and, indeed, shaded macaw seedlings showed the higher q_N values at the expenses of electron transport through PSII. Altogether, these phenomena reveal that the species modulated its physiology accordingly to the surroundings.

The higher F_v/F_m observed in shaded seedlings than in those exposed to sunlight over the entire evaluation period, is a common feature in plants grown under low irradiance¹², which maximizes light capture and promotes the efficient reduction of primary quinone electron acceptors in PSII⁴³. The homogeneous response of F_v/F_m

Treatments	Thickness of leaf tissues (μm)					
	Adaxial surface		Abaxial surface		Mesophyll	Leaf blade
	Epidermis	Hypodermis	Epidermis	Hypodermis		
Low light	39.52 \pm 0.94 a	63.18 \pm 3.88 a	44.45 \pm 2.63 a	61.93 \pm 6.21 a	326.42 \pm 24.26 a	523.60 \pm 35.84 a
High light	37.73 \pm 1.33 a	57.09 \pm 3.55 a	40.50 \pm 1.00 a	57.87 \pm 6.07 a	309.53 \pm 9.92 a	497.95 \pm 12.15 a

Table 2. Micromorphometric analysis of leaflets of young macaw palm plants grown in the long term under nursery conditions of low and high light. Means \pm standard error followed by the same letter in a column do not differ from each other according to Tukey's test at 5% significance level. (n = 5).

among the other treatments without significant changes, indicates that the sudden exposure to high light intensity did not cause photodamage to the leaves of macaw palm seedlings. In contrast, the transference from shade to full sunlight conditions was accompanied by photooxidation and reactive oxygen species (ROS) overproduction in shade-tolerant dicot tree species from the Atlantic forest in Brazil¹². In fact, no permanent photosynthetic damage occurred in macaw palm leaflets when transferred to high light, as corroborate the results on C_i/C_a and chlorophyll content index, besides F_v/F_m values. Chlorophyll molecules is very sensitive to photo-oxidative stress upon plant transference to excess light⁶, which may cause biochemical limitations of photosynthesis proven by increased C_i/C_a values. However, this was not observed for macaw palm seedlings exposed to sudden high light intensity. The capacity for avoiding damage on PSII mainly in low light plants transferred to high irradiance might be due to its efficiency in dissipating excess energy through non-photochemical processes. In addition, without damage, the increase in F_0 indicates a higher investment in a optimally organized and functional antenna system that are required for maximum q_N buildup⁴⁴. The punctual increases in F_0 observed in seedlings transferred to full sunlight after day 9 would indicate impairment in energy transfer from the antennae complex to the reaction center of the PSII. The decrease in F_v/F_m and increase in F_0 during the evaluation period would be the first symptoms of a leaf-age response⁴⁵, although no significant reductions in chlorophyll were observed (Supplementary Fig. S1).

The acclimation of the macaw palm to different light conditions was evidenced by the chlorophyll contents in the leaflets. Different levels of luminosity during the initial growth of the seedlings results in high levels of photosynthetic pigments because there is a decrease in the available light intensity^{10,46}. Nonetheless, the plants exposed to full sunlight maintained an efficient light capturing mechanism despite having lower chlorophyll contents than shaded plants did. The higher proportion of chlorophyll *b* in low light plants allows a greater uptake of light energy than in plants exposed to high light since the availability of this pigment influences the composition and size of the antenna complexes⁴⁷. In addition to chlorophyll *b*, carotenoids are accessory pigments that play an important role in the photoprotection of PSII under excessive light conditions⁴⁸. The high content of carotenoids in shaded macaw palm plants might have contributed to the effective non-photochemical energy dissipation. This mechanism involves the dissipation of excess energy that protects PSII reaction centers against light damage. Generally, leaves of plants grown in a shaded environment exhibited lower q_N than those grown under full sunlight conditions did^{46,49}. However, for macaw palms, the plants grown in the low light environment showed the highest q_N values, especially under high light intensities during the light curve (Fig. 3), which protected the photosynthetic apparatus. Consequently, the plants avoided photoinhibition, which might have reduced the rate of accumulation of ROS such as singlet oxygen and superoxide radical anions⁵⁰. This physiological adjustment contributed to the enhanced macaw palm growth under low irradiance condition, and the plants were able to establish themselves following exposure to high light intensity, such as conditions that occur with the presence of clearings in the forest canopy or cloudiness variation during the rainy season.

The excess energy dissipation in the photochemical apparatus depicted by q_N is the result of activation of various processes involved in the q_E , q_T and q_I ⁵¹. When we evaluated the three components of q_N (q_E , q_T , and q_I) in seedlings of macaw palm after long-term exposition to contrasting light intensities, the values of q_E under high light were greater than those under low light conditions, indicating that there was a greater dissipation of excess energy absorbed in the form of heat through the xanthophyll cycle. The xanthophylls are carotenoids involved in q_N , and they act as antioxidants and protect the antenna complex from damage by excessive light⁵⁰. While the q_T remained constant considering the high light exposure, the similar (non-significant) q_I in leaflets from plants in both treatments indicates that the photoprotective effect of the xanthophyll cycle was sufficient to prevent significant damage to the PSII even in shaded seedlings. It is noteworthy that the q_N partitioning in macaw palm was measured after exposure to excessive light levels that were above that necessary for photosynthetic saturation ($3000 \mu\text{mol m}^{-2} \text{s}^{-1}$), which resulted in relatively high q_I values⁴³.

The photochemical efficiency in contrasting light environments and the capacity to prevent photoinhibitory damage in macaw palm leaves was also demonstrated by the recovery of the properties after excess light treatment (Fig. 4). Moreover, activation of the fast mechanism for excess energy dissipation through q_E was efficient for a 65% recovery of the ϕ_{PSII} even after 1 min of high light exposure. However, 30 min in the dark was not sufficient to fully recover the maximum photochemical efficiency because of the photoinhibitory processes. Under excess irradiance photosynthesis may be severely inhibited, as observed with some vascular plant species^{7,8,49} including palms^{9,40}. During this process, the highest levels of ROS are observed, and there may be permanent damage to the photosynthetic apparatus, known as chronic photoinhibition^{50,52}. However, for the macaw palm, full recovery occurred 24 h after the excess light treatment, indicating the occurrence of dynamic photoinhibition, which is characterized by a temporary decrease in the maximum quantum efficiency of PSII⁵³ and suggests a protective mechanism that deals with high levels of irradiance. Moreover, the maintenance of the photochemical integrity

of the macaw palm under low light conditions was confirmed by the highest values of F_v/F_m found after the full recovery period. The ability to overcome possible photoinhibitory damage and the magnitude of the response to a sudden increase in light intensity vary among plant species and are dependent on their ability to acclimate to this new condition¹².

In addition to physiological adjustments, morphological changes also contribute to the acclimation responses to contrasting light conditions. The higher LA displayed during the initial growth of macaw palm seedling under the low light environment and its maximum photochemical efficiency, contribute to maximizing the light capture. However, during the transition from the pre-nursery stage to nursery-growing plants, with higher biomass accumulation²², the increase in the LA was similar between plants grown under contrasting light conditions, resulting in insignificant differences in the SLA. Generally, in dicot trees, shading increases the leaf height and LA compared to plants grown under full sunlight conditions^{8,10}. As observed for young macaw palm plants, the light intensity did not affect the growth of other palm species, such as *Acrocomia emensis*, *Butia paraguayensis*, and *Syagrus petraea*⁵⁴. The higher stipe length of the shaded macaw palm seedlings at the end of the assay may have been due to activation of some functional adjustments after long-term exposure to low light conditions⁵⁵.

In contrast to the other plant species¹⁰, macaw palm seedlings did not exhibit significant anatomical changes in leaf tissues following exposure to contrasting light intensities. In response to increased light intensity, the differentiation of new layers or stretching of the cells of the palisade parenchyma and consequently increase in the thickness of the mesophyll are common^{8,56}, as well as in the interception of light¹¹. However, the greater elongation of mesophyll cells on the adaxial surface of macaw palm leaflets than on other surfaces appears to be a typical structural pattern of the species, regardless of the light condition.

Macaw palm seedlings exhibited limited leaflet anatomical plasticity, but they adjust to contrasting light conditions by modulating their light capturing efficiency under low light, and maximizing photosynthetic efficiency and non-photochemical energy dissipation under excess light conditions. The ability to recover rapidly from photoinhibition after sudden exposure to high irradiances and the long-term adjustments in chlorophyll content and leaf morphological traits allowed macaw palm establishment and cultivation under extremely different light conditions.

References

- Smith, H. Light quality, photoperception, and plant strategy. *Annual Review of Plant Physiology* **33**, 481–518, <https://doi.org/10.1146/annurev.pp.33.060182.002405> (1982).
- Gommers, C. M. M., Visser, E. J. W., Onge, K. R. S., Voesenek, L. A. C. J. & Pierik, R. Shade tolerance: when growing tall is not an option. *Trends in Plant Science* **18**, 65–71, <https://doi.org/10.1016/j.tplants.2012.09.008> (2013).
- Dos Santos, M. L. S. *et al.* Low light availability affects leaf gas exchange, growth and survival of *Euterpe edulis* seedlings transplanted into the understory of an anthropic tropical rainforest. *Southern Forests: a Journal of Forest Science* **74**, 167–174, <https://doi.org/10.2989/20702620.2012.722833> (2012).
- Gatti, M. G., Campanello, P. I. & Goldstein, G. Growth and leaf production in the tropical palm *Euterpe edulis*: Light conditions versus developmental constraints. *Flora - Morphology, Distribution, Functional Ecology of Plants* **206**, 742–748, <https://doi.org/10.1016/j.flora.2011.04.004> (2011).
- Dickison, W. C. *Integrative plant anatomy*. (Academic Press, 2000).
- Dos Anjos, L. *et al.* Key leaf traits indicative of photosynthetic plasticity in tropical tree species. *Trees* **29**, 247–258, <https://doi.org/10.1007/s00468-014-1110-2> (2015).
- Fini, A. *et al.* Mesophyll conductance plays a central role in leaf functioning of Oleaceae species exposed to contrasting sunlight irradiance. *Physiologia Plantarum* **157**, 54–68, <https://doi.org/10.1111/ppl.12401> (2016).
- Sanches, M. C., Marzinek, J., Bragiola, N. G. & Terra Nascimento, A. R. Morpho-physiological responses in *Cedrela fissilis* Vell. submitted to changes in natural light conditions: implications for biomass accumulation. *Trees Struct. Funct.*, 1–13, <https://doi.org/10.1007/s00468-016-1474-6> (2016).
- Gatti, M. G., Campanello, P. I., Villagra, M., Montti, L. & Goldstein, G. Hydraulic architecture and photoinhibition influence spatial distribution of the arborescent palm *Euterpe edulis* in subtropical forests. *Tree Physiology* **34**, 630–639, <https://doi.org/10.1093/treephys/tpu039> (2014).
- Gaburro, T. A., Zanetti, L. V., Gama, V. N., Milanez, C. R. D. & Cuzzuol, G. R. F. Physiological variables related to photosynthesis are more plastic than the morphological and biochemistry in non-pioneer tropical trees under contrasting irradiance. *Brazilian Journal of Botany* **38**, 39–49, <https://doi.org/10.1007/s40415-014-0113-y> (2015).
- Tadrist, L. & Darbois-Textier, B. Are leaves optimally designed for self-support? An investigation on giant monocots. *Journal of Theoretical Biology* **396**, 125–131, <https://doi.org/10.1016/j.jtbi.2016.02.018> (2016).
- Dos Anjos, L., Oliva, M. & Kuki, K. Fluorescence imaging of light acclimation of Brazilian Atlantic forest tree species. *Photosynthetica* **50**, 95–108 (2012).
- Souza, F. M., Gandolfi, S. & Rodrigues, R. R. Deciduousness influences the understory community in a semideciduous tropical forest. *Biotropica* **46**, 512–515, <https://doi.org/10.1111/btp.12137> (2014).
- Machado, W., Figueiredo, A. & Guimarães, M. F. Initial development of seedlings of macauba palm (*Acrocomia aculeata*). *Industrial Crops and Products* **87**, 14–19, <https://doi.org/10.1016/j.indcrop.2016.04.022> (2016).
- Montoya, S. G., Motoike, S. Y., Kuki, K. N. & Couto, A. D. Fruit development, growth, and stored reserves in macauba palm (*Acrocomia aculeata*), an alternative bioenergy crop. *Planta* **244**, 927–938, <https://doi.org/10.1007/s00425-016-2558-7> (2016).
- Motta, P. D., Curi, N., Oliveira-Filho, A. D. & Gomes, J. B. V. Ocorrência da macaúba em Minas Gerais: relação com atributos climáticos, pedológicos e vegetacionais. *Pesquisa Agropecuária Brasileira* **37**, 1023–1031 (2002).
- Evaristo, A. B. *et al.* Actual and putative potentials of macauba palm as feedstock for solid biofuel production from residues. *Biomass and Bioenergy* **85**, 18–24 (2016).
- Pires, T. P., dos Santos Souza, E., Kuki, K. N. & Motoike, S. Y. Ecophysiological traits of the macaw palm: a contribution towards the domestication of a novel oil crop. *Industrial Crops and Products* **44**, 200–210, <https://doi.org/10.1016/j.indcrop.2012.09.029> (2013).
- Mota, C. S. & Cano, M. A. O. Matter accumulation and photosynthetic responses of macaw palm to cyclical drought. *Revista Caatinga* **29**, 850–858 (2016).
- Bicalho, E. M., Motoike, S. Y., Lima e Borges, E. E. D., Ataíde, G. D. M. & Guimarães, V. M. Enzyme activity and reserve mobilization during Macaw palm (*Acrocomia aculeata*) seed germination. *Acta Botanica Brasílica* **30**, 438–444 (2016).
- Motoike, S., Lopes, F., Sá Júnior, A., Carvalho, M. & Oliveira, M. Processo de germinação e produção de sementes pré-germinadas de palmeiras do gênero *Acrocomia*. *Patente: Submetido à Lei de Patentes. Protocolo INPI 1185103447* (2007).
- Pimentel, L. D., Bruckner, C. H., Manfio, C. E., Motoike, S. Y. & Martinez, H. E. P. Substrate, lime, phosphorus and topdress fertilization in macaw palm seedling production. *Rev. Arvore* **40**, 235–244 (2016).

23. Mazzanatti, T. *et al.* Light acclimation in nursery: morphoanatomy and ecophysiology of seedlings of three light-demanding neotropical tree species. *Brazilian Journal of Botany* **39**, 19–28, <https://doi.org/10.1007/s40415-015-0203-5> (2016).
24. Henderson, F. M. Morphology and anatomy of palm seedlings. *The Botanical Review* **72**, 273–329 (2006).
25. Genty, B., Briantais, J. M. & Baker, N. R. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta* **990**, 87–92 (1989).
26. Wellburn, A. R. The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *Journal of Plant Physiology* **144**, 307–313 (1994).
27. Baly, E. The kinetics of photosynthesis. *Proceedings of the Royal Society of London. Series B, Biological Sciences* **117**, 218–239 (1935).
28. Lobo, Fd. A. *et al.* Fitting net photosynthetic light-response curves with *Microsoft Excel* - a critical look at the models. *Photosynthetica* **51**, 445–456 (2013).
29. Hendrickson, L., Furbank, R. T. & Chow, W. S. A simple alternative approach to assessing the fate of absorbed light energy using chlorophyll fluorescence. *Photosynthesis Research* **82**, 73–81 (2004).
30. Kramer, D. M., Johnson, G., Kiirats, O. & Edwards, G. E. New fluorescence parameters for the determination of QA redox state and excitation energy fluxes. *Photosynthesis Research* **79**, 209–218 (2004).
31. Schreiber, U., Schliwa, U. & Bilger, W. Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynthesis research* **10**, 51–62 (1986).
32. Oxborough, K. & Baker, N. R. Resolving chlorophyll *a* fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components – calculation of q_p and F'_v/F'_m without measuring F'_o . *Photosynthesis Research* **54**, 135–142, <https://doi.org/10.1023/A:1005936823310> (1997).
33. Melis, A., Spangfort, M. & Andersson, B. Light-absorption and electron-transport balance between photosystem II and photosystem I in spinach chloroplasts. *Photochemistry and Photobiology* **45**, 129–136 (1987).
34. Zivcak, M., Brestic, M., Kalaji, H. M. & Govindjee Photosynthetic responses of sun- and shade-grown barley leaves to high light: is the lower PSII connectivity in shade leaves associated with protection against excess of light? *Photosynthesis Research* **119**, 339–354, <https://doi.org/10.1007/s1120-014-9969-8> (2014).
35. Lichtenthaler, H. K., Buschmann, C. & Knapp, M. How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio RFD of leaves with the PAM fluorometer. *Photosynthetica* **43**, 379–393, <https://doi.org/10.1007/s11099-005-0062-6> (2005).
36. Johansen, D. A. Plant microtechnique. *Plant microtechnique* (1940).
37. O'Brien, T., Feder, N. & McCully, M. E. Polychromatic staining of plant cell walls by toluidine blue O. *Protoplasma* **59**, 368–373 (1964).
38. Demmig-Adams, B., Cohu, C. M., Muller, O. & Adams, W. W. Modulation of photosynthetic energy conversion efficiency in nature: from seconds to seasons. *Photosynthesis Research* **113**, 75–88, <https://doi.org/10.1007/s1120-012-9761-6> (2012).
39. Apichatmeta, K., Sudsiri, C. J. & Ritchie, R. J. Photosynthesis of oil palm (*Elaeis guineensis*). *Scientia Horticulturae* **214**, 34–40, <https://doi.org/10.1016/j.scienta.2016.11.013> (2017).
40. Araus, J. L. & Hogan, K. P. Leaf structure and patterns of photoinhibition in two neotropical palms in clearings and forest understory during the dry season. *American Journal of Botany*, 726–738 (1994).
41. Skillman, J. B. Quantum yield variation across the three pathways of photosynthesis: not yet out of the dark. *Journal of Experimental Botany* **59**, 1647–1661 (2008).
42. Rozendaal, D., Hurtado, V. & Poorter, L. Plasticity in leaf traits of 38 tropical tree species in response to light; relationships with light demand and adult stature. *Functional Ecology* **20**, 207–216 (2006).
43. Baker, N. R. Chlorophyll fluorescence: a probe of photosynthesis *in vivo*. *Annu. Rev. Plant Biol.* **59**, 89–113 (2008).
44. Lokstein, H., Tian, L., Polle, J. E. W. & DellaPenna, D. Xanthophyll biosynthetic mutants of *Arabidopsis thaliana*: altered nonphotochemical quenching of chlorophyll fluorescence is due to changes in Photosystem II antenna size and stability. *Biochimica et Biophysica Acta-Bioenergetics* **1553**, 309–319 (2002).
45. Juvany, M., Müller, M. & Munné-Bosch, S. Photo-oxidative stress in emerging and senescing leaves: a mirror image? *Journal of experimental botany* **64**, 3087–3098 (2013).
46. Hazrati, S., Tahmasebi-Sarvestani, Z., Modarres-Sanavy, S. A. M., Mokhtassi-Bidgoli, A. & Nicola, S. Effects of water stress and light intensity on chlorophyll fluorescence parameters and pigments of *Aloe vera* L. *Plant Physiology and Biochemistry* **106**, 141–148, <https://doi.org/10.1016/j.plaphy.2016.04.046> (2016).
47. Voitsekhovskaja, O. V. & Tyutereva, E. V. Chlorophyll *b* in angiosperms: functions in photosynthesis, signaling and ontogenetic regulation. *Journal of Plant Physiology* **189**, 51–64, <https://doi.org/10.1016/j.jplph.2015.09.013> (2015).
48. Havaux, M. & Niyogi, K. K. The violaxanthin cycle protects plants from photooxidative damage by more than one mechanism. *Proceedings of the National Academy of Sciences* **96**, 8762–8767 (1999).
49. Costa, A. C. *et al.* The effect of irradiance and water restriction on photosynthesis in young jatobá-do-cerrado (*Hymenaea stigonocarpa*) plants. *Photosynthetica* **53**, 118–127, <https://doi.org/10.1007/s11099-015-0085-6> (2015).
50. Kreslavski, V. D., Zorina, A. A., Los, D. A., Fomina, I. R. & Allakhverdiev, S. I. In *Molecular Stress Physiology of Plants*, 21–51 (2013).
51. Walters, R. G. & Horton, P. Resolution of components of non-photochemical chlorophyll fluorescence quenching in barley leaves. *Photosynthesis Research* **27**, 121–133 (1991).
52. Osmond, C. B. What is photoinhibition? Some insights from comparisons of shade and sun plants. *Photoinhibition of Photosynthesis from Molecular Mechanisms to the Field* (1994).
53. Werner, C., Correia, O. & Beyschlag, W. Characteristic patterns of chronic and dynamic photoinhibition of different functional groups in a Mediterranean ecosystem. *Functional Plant Biology* **29**, 999–1011, <https://doi.org/10.1071/PP01143> (2002).
54. Amadeu, L. S. N., Sampaio, M. B. & dos Santos, F. A. M. Influence of light and plant size on the reproduction and growth of small palm tree species: Comparing two methods for measuring canopy openness. *American Journal of Botany* **103**, 1678–1686, <https://doi.org/10.3732/ajb.1600178> (2016).
55. Coble, A. P. & Cavaleri, M. A. Light acclimation optimizes leaf functional traits despite height-related constraints in a canopy shading experiment. *Oecologia* **177**, 1131–1143, <https://doi.org/10.1007/s00442-015-3219-4> (2015).
56. Calzavara, A. K. *et al.* Morphoanatomy and ecophysiology of tree seedlings in semideciduous forest during high-light acclimation in nursery. *Photosynthetica* **53**, 597–608, <https://doi.org/10.1007/s11099-015-0151-0> (2015).

Acknowledgements

The authors thank the National Council for Scientific and Technological Development CNPq (470116/2013-7) and the Foundation for Research Support of Minas Gerais - FAPEMIG (CRA-APQ-01244-13) for funding the research. The authors also thank FAPEMIG for the Master's grant awarded to A.N. Dias and the postdoctoral grant awarded to A.I. Siqueira-Silva (CRA-BPD-00618-14). The authors thank Alessandro D. Halfeld for technical assistance and acknowledge the Acrotech Sementes e Reflorestamento Ltda company for providing the macaw palm seeds. E.G. Pereira also thanks CNPq for the research productivity grant (311532/2017-9).

Author Contributions

E.G.P. and K.N.K. conceived and designed the study. A.N.D. performed the experiments and together with E.G.P. performed the statistical analysis. E.G.P., A.I.S.-S. and J.P.S. interpreted the results, and together with K.N.K. wrote the manuscript.

Additional Information

Supplementary information accompanies this paper at <https://doi.org/10.1038/s41598-018-33553-1>.

Competing Interests: The authors declare no competing interests.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2018