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ORIGINAL ARTICLE

Light spectrum and explant type drive hormonal and proteomic reprogramming in micropropagation of the endangered *Paubrasilia echinata*

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Abstract

Paubrasilia echinata is an endangered and emblematic tree species of the Brazilian Atlantic Forest, requiring efficient biotechnological strategies for its conservation. This study aimed to establish optimal conditions for in vitro propagation of *P. echinata* by evaluating the effects of explant type and light spectrum on shoot development, as well as to characterize the biochemical and proteomic alterations associated with shoot development. Cotyledonary nodal segments incubated under light-emitting diode (LED) lamp with a combined light spectrum of white, medium blue, deep red, and far-red (W/mB/dR/fR) exhibited the greatest shoot elongation and biomass accumulation. The LED W/mB/dR/fR lamp induced distinct metabolic reprogramming associated with shoot development, leading to increased endogenous levels of benzyladenine, abscisic acid, and putrescine. Furthermore, enhanced shoot growth under LED W/mB/dR/fR was associated with the up-accumulation of proteins involved in photosynthesis (oxygen-evolving enhancer protein 1), redox homeostasis (ascorbate peroxidase and peroxidases), cellular stability (heat shock proteins), and cytoskeletal organization (tubulin). Ex vitro rooting ranged from 50–65% and was not significantly influenced by explant type or indole-3-butyric acid concentration. Overall, these findings establish a physiologically supported micropropagation protocol for large-scale production of *P. echinata*, providing a strategic tool for the conservation of this culturally and ecologically Brazilian species.

Key message

Optimized light spectrum and explant type enhance shoot development and trigger hormonal and proteomic reprogramming in *Paubrasilia echinata*, enabling a physiologically supported micropropagation protocol for conservation of this endangered species.

Keywords In vitro propagation · LED lamp · *Caesalpinia echinata* · Red light spectrum · Polyamines · Plant growth hormones · Proteomics

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Introduction

Paubrasilia echinata (Lam.) E. Gagnon, H.C. Lima & G.P. Lewis (Fabaceae), commonly known as Pau-brasil, is an endemic and emblematic tree species of the Brazilian Atlantic Forest (Esser et al. 2019; SOS Mata Atlântica & INPE 2024). Owing to its exceptional historical and cultural relevance, having given Brazil its name and being declared the national tree by Federal Law No. 6,607 (Brasil 1978). The species has long been subjected to intense exploitation. As a consequence of overharvesting and habitat loss, *P. echinata* is classified as Endangered on the Union for Conservation of Nature on the Red List (IUCN 2025), reinforcing the need for efficient conservation and propagation strategies.

Although vegetative propagation of *P. echinata* has been reported, important limitations persist. Seeds require specific storage conditions to maintain viability (Hellmann et al. 2006; Mello et al. 2013), while vegetative propagation via cuttings exhibits low rooting efficiency, even when treated with high auxin concentrations (Endres et al. 2007). Given these constraints, in vitro propagation using nodal segments represents a promising biotechnological alternative, enabling clonal multiplication and supporting conservation programs for threatened woody species.

In vitro morphogenesis is regulated by multiple factors, including explant type, plant growth regulators, and environmental conditions (Pulianmackal et al. 2014). Cytokinins, particularly Benzyladenine (BA), play a central role on shoot development from axillary buds of nodal segments (Ivanova et al. 2006; Aragão et al. 2016; Pessanha et al. 2022). However, morphogenic responses are highly species and explant-dependent. In several woody species, cotyledonary nodal segments exhibit greater morphogenic competence than apical, likely due to differences in developmental stage and endogenous hormonal balance (Aragão et al. 2016; Lerin et al. 2019; Rodrigues et al. 2025).

Beyond chemical regulation, light quality is a key physical factor influencing in vitro morphogenesis. The use of light-emitting diode (LED) technology has enabled precise control of light spectra, allowing targeted modulation of photomorphogenesis, shoot elongation, and biomass accumulation (Fan et al. 2022). Combinations of blue, red, and far-red wavelengths from LED lamps have consistently promoted superior shoot development in woody species when compared to fluorescent lamp (Lerin et al. 2019; Oliveira et al. 2020; Silva et al. 2020; Rodrigues 2023). These light-induced responses are often associated with changes in endogenous polyamines (PAs) and phytohormones, which play crucial roles in cell division, elongation, and stress responses (Silveira et al. 2004; Ali et al. 2024).

PAs, particularly putrescine (Put), are closely linked with in vitro shoot development and are known to be modulated by light quality (Lerin et al. 2019; Oliveira et al. 2020). In parallel, phytohormones, including auxins, cytokinins, abscisic acid (ABA), salicylic acid (SA), and jasmonates, act in an integrated and coordinated manner to regulate morphogenesis, with the balance between auxin and cytokinin being especially critical for shoot formation and development (Skoog and Miller 1957; Asghar et al. 2023). Previous studies in woody species have demonstrated that alterations in endogenous hormonal profiles can directly impact shoot growth and rooting capacity during successive subcultures (Oliveira et al. 2022).

In addition to biochemical analyses, proteomic approaches have emerged as powerful tools to elucidate the molecular mechanisms underlying in vitro morphogenesis. Differential protein accumulation related to photosynthesis, metabolism, redox homeostasis, stress responses, and cytoskeletal organization has been associated with improved shoot development under specific light spectra and explant types (Lerin et al. 2019; Oliveira et al. 2020; Pessanha et al. 2022). These studies highlight the importance of integrating physiological, biochemical, and proteomic data to better understand shoot development in vitro.

Despite significant advances in micropropagation and omics-based analyses in several woody species, such integrated approaches remain largely unexplored for *P. echinata*. Therefore, this study aimed to establish optimal conditions for the in vitro propagation of *P. echinata* by evaluating the effects of explant type and light spectrum on shoot development, as well as to characterize the associated biochemical and proteomic reprogramming. Additionally, the efficiency of ex vitro rooting was assessed as a complementary step toward complete plantlet production.

Material and methods

Plant material

Immature fruits of *P. echinata* were collected six weeks after flowering from trees located in Campos dos Goytacazes, Rio de Janeiro, Brazil (21°45′43″S, 41°17′28″W), to obtain seeds used for in vitro germination. Specimens of *P. echinata* were deposited in the Herbarium of the Universidade Estadual do Norte Fluminense Darcy Ribeiro (HUENF 7483, HUENF 7500, HUENF 8126, HUENF 9618; https://jabot.jbrj.gov.br/v3/consulta.php).

Fruit disinfection and seed germination

Fruits were surface disinfected according to Santa-Catarina et al. (2001). First, fruits were washed in water containing one drop of neutral detergent (Limpol; São Paulo, Brazil),

immersed in 70% ethanol (Tupi; São Paulo, Brazil) for one min, and subsequently treated with 100% commercial bleach (Qboa®; Anhembi, São Paulo - Brazil) containing 1.8–2.5% sodium hypochlorite for 1 h. Finally, the fruits were rinsed three times with autoclaved type II deionized water in a flow chamber. Immature seeds were isolated from disinfected fruits and germinated in wood plant medium (WPM; PhytoTech Labs®, Kansas, USA) (Lloyd and McCown 1980), both supplied with 20 g L^{-1} sucrose (Synth; São Paulo, Brazil), 2 g L^{-1} gellan gum (Phytagel®; Sigma–Aldrich; St Louis, USA). The pH of culture media was adjusted to 5.7 before Phytagel® addition. The culture medium was then distributed into test tubes (10 mL) and autoclaved at 121 °C for 20 min. After transferring to the culture medium, seeds were kept in a growth room with a light intensity of 73 µmol.$m^{-2}.s^{-1}$, an 16-h light photoperiod, at 25 ± 2 °C.

Effect of explant type and BA concentration on shoot development

Apical and cotyledonary nodal segments (±1.5 cm) were isolated from 60 day-old-seedlings (Fig. 1) and transferred in WPM (PhytoTech Labs®) culture medium supplemented with 20 g L^{-1} sucrose (Synth), 2 g L^{-1} Phytagel® (Sigma–Aldrich), and different concentrations (0, 0.1, 0.5, and 1.0 µM) of BA (Sigma–Aldrich). The pH of the culture medium was adjusted to 5.7, and then Phytagel® was added. The culture medium was then distributed into culture flasks (30 mL) and autoclaved at 121 °C for 20 min. After transferring to the culture medium, the explants were kept in a growth room with a light intensity of 73 µmol.$m^{-2}.s^{-1}$, an 8-h photoperiod, at 25 ± 2 °C. Each treatment was composed by eight replicates, with five explants per replicate. After 120 days, the induction (%), shoot length (cm), and number of shoots per explant were evaluated.

Effects of explant type and light spectra on shoot development

Apical and cotyledonary nodal segments (±1.5 cm) from 60-day-old seedlings in vitro germinated were excised and transferred to WPM (PhytoTech Labs®) culture medium supplemented with 20 g L^{-1} sucrose (Synth), 0.1 µM BA (Sigma-Aldrich), and 2 g L^{-1} Phytagel® (Sigma–Aldrich). The pH of the culture medium was adjusted to 5.7 before adding Phytagel®, dispensed into flasks (30 mL) and autoclaved at 121 °C for 20 min. Explant segments were cultured under seven light treatments, being six LED lamps and one fluorescent lamp as a control (Table 1). The flasks were covered with four layers of shade cloth, reducing the light

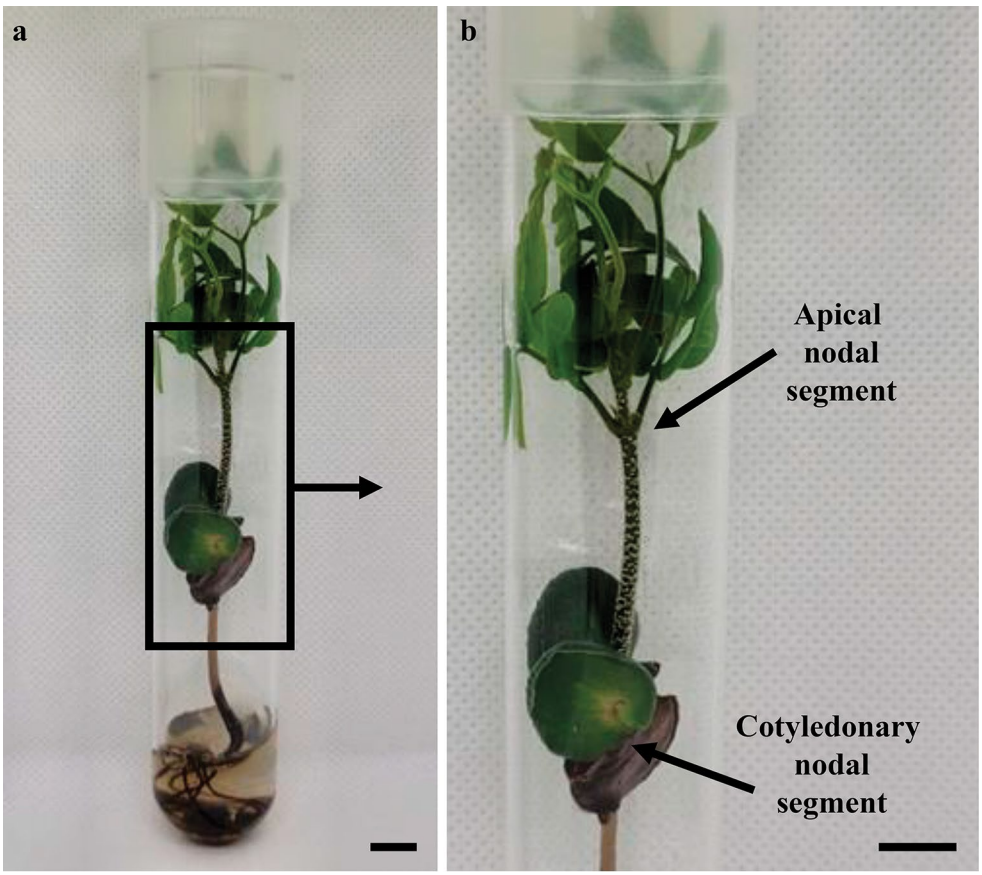


Fig. 1 Sixty-day-old seedlings (**a**) of *Paubrasilia echinata* in vitro germinated, showing the apical and cotyledonary nodal segments (**b**) used as explants for in vitro shoot development. Bars = 1 cm

Table 1 Characteristics of the different LED light wavelengths and tubular fluorescent lamp used in *Paubrasilia echinata* shoots development

White (W); low blue (LB) = 8–10% blue; medium blue (MB) = 12–14% blue; high blue (HB) = 16–18% blue; deep red (DR) = 30–50% deep red; far red (FR) = 12% far red

Lamps	Lamp wavelenght	Luminous intensity at the culture flask (μmol $m^{-2}s^{-1}$)	Peak wavelength (nm)
Control	Tubular fluorescent	16	435/545/580
WmB	W/MB	16	450/530
WhB	W/HB	16	450/530
WlBdR	W/LB/DR	16	450/530/660
WmBdR	W/MB/DR	16	450/530/660
WlBdRfR	W/LB/DR/FR	16	450/530/660/735
WmBdRfR	W/MB/DR/FR	16	450/530/660/735

intensity from 73 to 16 μmol $m^{-2}.s^{-1}$ at 25 °C. Each treatment consisted of eight replicates, with each replicate being one culture flask containing five explants. After 120 days, the induction (%), shoot length (cm), and number of shoots per explant were recorded. Fresh weight (FW), photosynthetic pigments, PA, phytohormones, and proteomic analyses were performed on samples from the fluorescent lamp (control) and the LED lamp that promoted the greatest shoot elongation. Samples for PA, phytohormone, and proteomic analyses were collected and stored at − 80 °C until further analysis.

Effect of IBA on shoot rooting

The rooting of shoots from cotyledonary and apical shoots was carried out ex vitro according to Ribeiro et al. (2022). Shoot cuttings (1.5 to 2.0 cm) were isolated and the base was immersed in different concentrations (0, 100, 250 and 500 μM) IBA for 3 h. Then, the shoot cuttings were then transferred to plastic cups (50 mL; TotalPlast, Santa Catarina, Brazil) containing the substrate Basaplant® (São Paulo, Brazil) and vermiculite (Basil Minérios; Goiás, Brazil) (1:1;v/v) and maintained in plastic trays (39.4 × 31.9 × 15.4 cm) (Pleion; São Paulo, Brazil) covered with PVC-type plastic film (Lumipam; São Paulo, Brazil) to maintain high relative humidity. The trays were maintained in a growth room at 25 ± 2 °C under a photoperiod of 16 h, with a light intensity of 55 μmol m^{-2} s^{-1} provided by LED lamps (Koninklijke Philips Electronics NV). After 25 days, aiming to reduce the moisture inside trays and stimulate the start of an acclimatization process simultaneously with rooting induction, the PVC parafilm plastic was perforated. This procedure was carried out until the complete removal of the PVC at 100 days after the start of perforation. After 240 days, the rooted plantlets (%) were evaluated.

Rooted plantlets were transferred to plastic bags containing soil and maintained in a greenhouse, receiving irrigation three times daily (6:00, 10:00, and 17:00 h) during 210 days. The micropropagated plantlets were transplanted to a field in a biotechnological trial at Instituto Biasse Socioambiental (https://institutobiasse.org.br/) located in Itaocara – RJ – Brazil (21°40′05,18″S, 42°03′32,62″W), for environmental education and extension activities.

FW determination

The FW of shoots developed under fluorescent and the LED treatment that promoted the higher shoot development from apical and cotyledonary nodal segments was determined using a precision balance. Five repetitions, with a culture flask containing five shoots each repetition, were used for FW analysis.

Proteomic analysis

For comparative proteomic analysis, samples (300 mg FW each, in triplicate) of shoots were collected from apical and cotyledonary nodal segments after 120 days of incubation under two lighting conditions: the LED lamp that promoted the greatest shoot elongation, and fluorescent lamp used as the control. Initially, samples were ground into a fine powder in liquid nitrogen. Proteins were extracted using a modified trichloroacetic acid (TCA)/acetone method (Damerval et al. 1986). The protein concentration was determined using the 2-D Quant Kit (Cytiva).

Before trypsin (V5111; Promega, Madison, USA; final enzyme-to-protein ratio of 1:100) digestion, proteins were precipitated using the methanol/chloroform method (Nanjo et al. 2012). Following precipitation, the samples were resuspended in a 7 M urea/2 M thiourea solution. Aliquots containing 100 μg of protein were digested according to the filter-aided sample preparation (FASP) protocol (Wiśniewski et al. 2009). Peptides were then resuspended and quantified at 205 nm using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific; Waltham, USA). Samples were transferred to Total Recovery Vials (Waters; Manchester, UK) for mass spectrometry.

Mass spectrometry analyses were performed using a nanoACQUITY UPLC system coupled to a Synapt G2-Si HDMS mass spectrometer (Waters) operated in nano-electrospray liquid chromatography tandem mass spectrometry mode (nanoESI-LC–MS/MS), and spectral processing

and database searches were performed using ProteinLynx Global SERVER (PLGS) software (version 3.0.2; Waters) according to Carrari-Santos et al. (2024), against the *P. echinata* (Lam.) protein database. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (Perez-Riverol et al. 2025) partner repository with the dataset identifier PXD070163. Label-free quantification analyses were performed using ISOQuant software v. 1.7 (Distler et al. 2014).

Statistical comparisons were performed using two-tailed Student's t-test. Proteins with $p\text{-}value < 0.05$ were classified as up-accumulated if the Log_2 fold-change (FC) exceeded 0.6, and down-accumulated if Log_2 (FC) was below −0.6. Functional annotations were performed using OmicsBox v0.3.0.29 (https://www.biobam.com). Protein sequences were queried against the NCBI non-redundant green plant protein database (taxa: 33,090; Viridiplantae) using BLAST. The enrichment analysis of KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways of differentially accumulated proteins (DAPs) was performed in STRING version 12.0 (https://string-db.org) using *Glycine max* homologs of *P. echinata* proteins. To generate a protein–protein interaction (PPI) network, *G. max* was considered the reference plant species using a minimum required interaction score of 0.7, and network analysis was performed with Cytoscape (https://cytoscape.org) (version 3.10.2) (Shannon et al. 2003).

Analysis of photosynthetic pigments

Photosynthetic pigments chlorophyll (Chl) a, b, and carotenoids were analyzed according to Arnon (1949). Samples (200 mg FW, in triplicate) of shoots from apical and cotyledonary nodal segments after 120 days of incubation under LED and fluorescent lamps were used. The photosynthetic pigments were extracted with 10 mL of 80% acetone (Sigma-Aldrich). Subsequently, the samples were incubated in the dark at 3 °C for 24 h. Absorbance readings were performed at wavelengths of 663 nm for Chl a, 645 nm for Chl b, and 470 nm for carotenoids, using the SoftMax Pro® 6.0 software. Pigment content was calculated using the following equations (Arnon 1949):

$$Chla = \frac{(12.7 \times OD_{663} - 2.69 \times OD_{645}) \times V}{1000 \times W}$$

$$Chlb = \frac{(22.9 \times OD_{645} - 4.68 \times OD_{663}) \times V}{1000 \times W}$$

$$Carotenoids = \frac{(1000 \times OD_{470} - 3.27 \times Chla - 104 \times Chlb)}{229 \times 1000 \times W}$$

Where: OD represents the optical density, V is the total extract volume (mL), and W is the FW (g) of the shoots.

Determination of free PAs content

For free PAs analysis, shoots originating from apical and cotyledonary nodal segments after 120 days of incubation under the LED lamp that promoted the highest shoot elongation and under the fluorescent lamp (control) were used. Free PAs were determined following the methodology of Silveira et al. (2004). Samples (200 mg FW each, in triplicate) of shoots were macerated with 1.2 mL of 5% perchloric acid (Merck Millipore; Darmstadt, Germany), incubated for 1 h at 4 °C, and centrifuged at 20,000 × g for 20 min at 4 °C. The supernatant was collected, and free PAs were determined directly from the supernatant by derivatization with dansyl chloride (Merck Millipore). The dansylated PAs were partitioned with toluene (Merck Millipore), which was then evaporated under nitrogen, and the residues were resuspended in absolute acetonitrile (Merck Millipore). Samples were analyzed by high-performance liquid chromatography (HPLC; Shimadzu, Kyoto, Japan) on a reverse-phase C18 column (Shimadzu Shin-pack CLC ODS). The mobile phase gradient was generated by mixing increasing volumes of absolute acetonitrile with 10% aqueous acetonitrile (pH 5.4, adjusted with HCl). The gradient was programmed from 0 to 65% acetonitrile over the first 10 min, from 65 to 100% between 10 and 13 min, and maintained at 100% from 13 to 21 min, at a flow rate of 1 mL/min and a column temperature of 40 °C. PA peaks were detected using a fluorescence detector (excitation 340 nm, emission 510 nm). The peak areas and retention times of the PAs were measured by comparison with those of standard PAs: Put, spermidine (Spd) and spermine (Spm) (Sigma-Aldrich).

Determination of plant hormones

The plant hormones indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), BA, ABA, gibberellic acid (GA), SA, jasmonic acid (JA) and the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) were extracted according to Xavier et al. (2025), with modifications. Samples (200 mg FW each, in quintuplicate) of 120-day-old shoots from cotyledonary nodal segments were collected, frozen in liquid nitrogen, and grounded into a fine powder. Then, 1.5 mL of extraction buffer composed of 80% ethanol (Sigma-Aldrich) and 1% polyvinylpyrrolidone (PVP-40) (Sigma–Aldrich) was added. Subsequently, the samples were sonicated for 10 min and centrifuged at 4 °C for 10 min. The supernatant was collected and vacuum-dried at 45 °C for 140 min until it reached approximately 200 µL. The sample volume was then adjusted with MS water (MS

grade; Tedia) to 500 µL 500 mg^{-1} (using a scale, considering 1:1 mass and volume), and the pH was corrected to 2.5 to 3.2 with 40 µL of acetic acid (Synth, São Paulo, Brazil). To each sample, 1 mL of ethyl ether (Sigma–Aldrich) was added and incubated on ice for 2 min, collecting the organic phase in a new microtube and placing it in a vacuum concentrator at 45 °C until the samples were completely dried. The samples were reconstituted in 500 µL of methanol:MS water (10:90), filtered using a syringe with a PTFE 0.22 µm filter (Merck Millipore; Darmstadt, Germany), and stored in vials (Waters; Manchester, UK).

The hormones were separated by liquid chromatography using 10 µL of the solution recovered from the extraction on a heated BEH C18 1.7 µm column (2.1 µm × 50 mm; Waters) at 40 °C, with a flow rate of 300 µL min^{-1}. The binary gradient consisted of LC-MS grade water with 0.01% formic acid and LC-MS grade methanol with 0.01% formic acid as the eluent. The elution gradient started at 10% methanol and was maintained for two minutes, increasing linearly to 90% over four minutes, then increasing linearly to 100% over one minute, and decreasing to 10% over 50 s, held at 10% for 70 s, totaling a nine-min run.

Using a high-performance electrospray ionization (ESI) source with dual orthogonal Z-spray (Waters), the effluents from the Acquity UPLC I-Class FTN (Waters) were introduced into the Xevo TQ-XS triple quadrupole mass spectrometer (Waters). Pressurized nebulization of nitrogen gas at 7 Bar, with a desolvation flow rate of 1000 L h^{-1} and a cone gas flow of 150 L h^{-1}, was employed. The desolvation gas temperature was set at 650 °C, the source temperature at 150 °C, and the capillary voltage at 3 kV. For the operation of the tandem dual quadrupole (MS/MS), argon gas was used as the collision gas at a pressure of 0.8 kgf cm^2 in the collision cell. The spectrometer operated in multiple reaction monitoring (MRM) mode, with cone voltage, collision energy, precursor mass, and fragment mass depending on the hormone under study. MassLynx software v0.4.2 (Waters) was used to process the chromatograms. Following acquisition, the spectra were integrated, and hormones were quantified using TargetLynx XS software (Waters). The area under the peak of each hormone was compared with a standard curve from each plant hormone (IAA, IBA, BA, ABA, GA, SA, JA, ACC).

Data analysis

All data were subjected to analysis of variance (ANOVA). Prior ANOVA, the Shapiro–Wilk test was applied to assess the normality of the data. Mean values for shoot variables, FW, PAs, and photosynthetic pigments were compared using Tukey's multiple comparison test at a 5% significance level, whereas plant hormone means were compared using a Student's *t*-test ($p \leq 0.05$). All statistical analyses were performed in R software version 4.4.2 R Development Core Team R (2024).

Results

Effects explant type and BA concentrations on shoot development

The interaction between explant type and BA concentration was not significant according to the two-way ANOVA for shoot length, number of shoots per explant, as well as shoot induction (%). However, the main effects of explant type and BA concentration were significant. For the type of explant, cotyledonary nodal segments produced shoots with greater elongation (Fig. 2a), as well as higher percentage of shoot induction (Fig. 2b) and number of shoots (Fig.

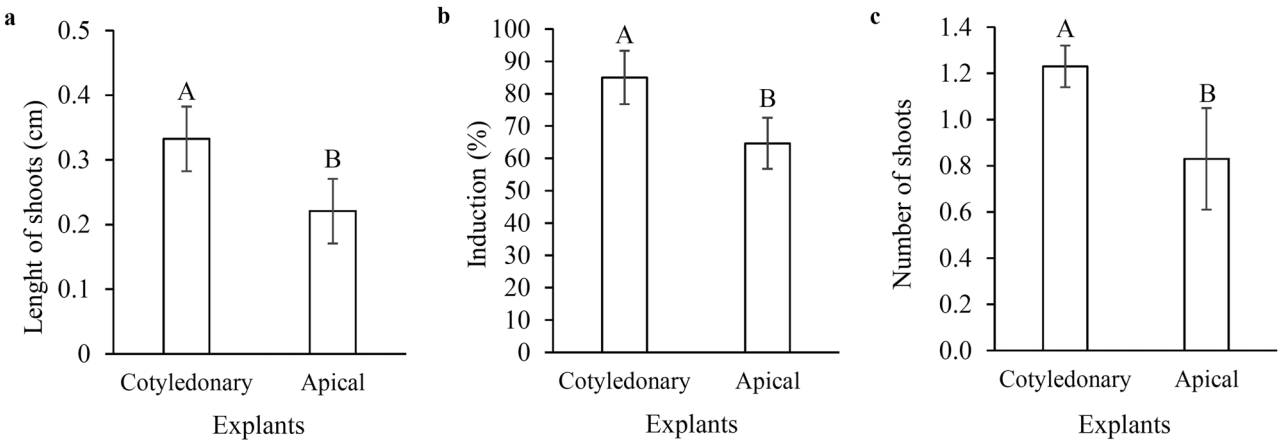


Fig. 2 Length (**a**), induction (**b**) and number (**c**) of shoots in *Paubrasilia echinata* from apical and cotyledonary nodal segments after 120 days of in vitro incubation. Means followed by different letters indicate significant differences at the 5% probability level according to Tukey's test. CV = coefficient of variation (mean ± standard deviation; $n = 8$; CV a = 35.98%; CV b = 26.83%; CV c = 32.93%)

2c) compared to apical nodal segments. Although 0.1 µM BA promoted longer shoots, no significant difference was observed compared to the control treatment (Fig. 3a). In contrast, higher BA concentrations (0.5 and 1 µM) reduced significantly the shoot induction (Fig. 3b) and number of shoots per explant (Fig. 3c).

Effects of explant type and light spectra on shoot development

LED light treatments had a significant effect on elongation of shoots from both apical and cotyledonary nodal segments compared to shoots grown under a fluorescent lamp (Figs. 4 and 5). The greatest elongation of shoots from cotyledonary nodal segments (1.70 cm) was observed under the LED lamp W/mB/dR/fR, which combined white (W), medium blue (mB), deep red (dR), and far red (fR) spectra, compared to the fluorescent lamp (0.85 cm), not differing significantly from shoots maintained under the LED lamp W/lB/dR/fR, with W, low blue (lB), dR, and fR spectra (Fig. 4a). Moreover, cotyledonary nodal segments exhibited greater elongation than those from apical segments, except under the LED lamp W/mB/dR with W, mB and dR, where no significant difference was observed between explant types. The number (Fig. 4b) and induction (Fig. 4c) of shoots were not significantly influenced by either the LED lamp treatment or explant type.

Shoots from cotyledonary nodal segments under the LED W/mB/dR/fR lamp exhibited significantly higher FW than shoots from apical segments. However, no significant differences were found between the two explant types under the fluorescent lamp. Additionally, FW did not differ between apical and cotyledonary explants under fluorescent lamp. However, under LED conditions, cotyledonary nodal segments showed significantly higher fresh weight than apical segments. No significant differences were observed between light sources for the same explant type (Fig. 6).

Effect of explant type and spectra light on proteomic profile

Comparative proteomic analysis was performed using shoots derived from cotyledonary and apical nodal segments grown under LED W/mB/dR/fR and fluorescent lamps (Fig. 7). Comparing shoots from cotyledonary nodal segment grown under LED W/mB/dR/fR and fluorescent lamp (CLED/CFLU comparison), a total of 31 proteins were identified, in which 25 of which were DAPs, including 1 unique to shoots grown in LED (CLED) and 2 unique proteins in shoots grown under fluorescent lamp (CFLU) (Fig. 7a and b).

Comparing shoots from apical nodal segment grown under LED W/mB/dR/fR and fluorescent (FLU) lamp (ALED/AFLU comparison), a total of 39 proteins were identified, 22 of which were DAPs, including 1 unique protein in shoots from Apical segments grown under LED (ALED) and 2 unique proteins in shoots grown under fluorescent lamp (AFLU; Fig. 7c and d). Comparing shoots from Cotyledonary nodal segments (C) with Apical nodal (A) under LED incubation (comparison CLED/ALED), a total of 20 proteins were identified, 19 of which were DAPs, including 1 unique to shoots from apical segments in LED W/mB/dR/fR lamp (ALED; Fig. 7e and 7f).

In the comparison of shoots from cotyledonary with those from apical nodal segments grown under fluorescent (FLU) lamp (CFLU/AFLU comparison) a total of 20 proteins were

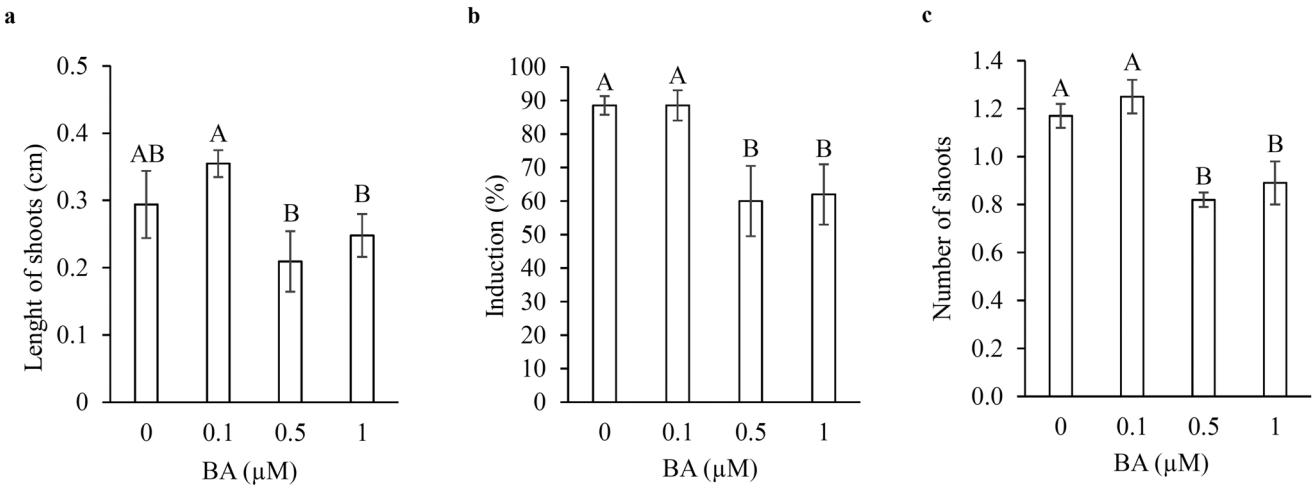


Fig. 3 Length (**a**), induction (**b**) and number (**c**) of shoots in *Paubrasilia echinata* after 120 days of in vitro incubation in different concentrations of BA. Data from apical and cotyledonary nodal segments were pooled and analyzed together. Means followed by different letters show significant differences at the 5% probability level according to Tukey's test. CV = coefficient of variation. (mean ± standard deviation; $n = 8$; CV a = 35.98%; CV b = 26.83%; CV c = 32.93%)

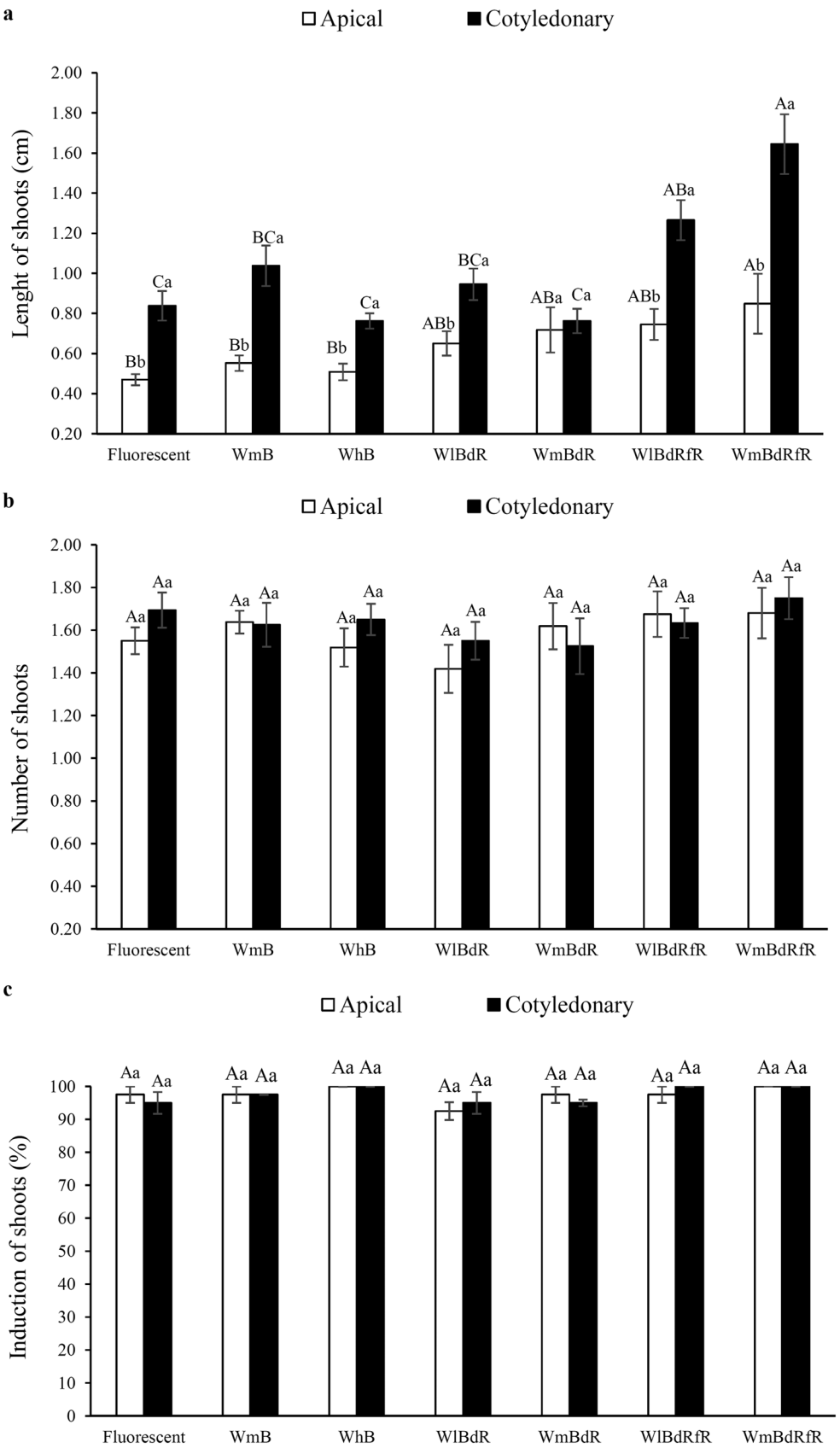


Fig. 4 Length (**a**), number (**b**), and induction (**c**) of shoots in *Paubrasilia echinata* after 120 days of in vitro incubation under different LED and fluorescent lamps: fluorescent; LED WmB = white (W) and medium blue (mB); LED WhB = white (W) and higher blue (hB); WlBdR = white (W), low blue (lB) and deep red dR; LED WmBdR = white (W), medium blue (mB) and deep red (dR); LED WlBdRfR = white (W), low blue (lB), deep red (dR) and far-red (fR); and LED WmBdRfR = white (**w**), medium blue (mB), deep red (dR) and far-red (fR). Means followed by different letters indicate significant differences at the 5% probability level according to Tukey's test. Capital letters indicate significant differences for each type of explant (apical or cotyledonary nodal segments) among different lamp treatments. Lowercase letters indicate significant differences between two types of explants (apical and cotyledonary nodal segments) within the same lamp treatment. CV = coefficient of variation. (mean ± standard deviation; $n = 8$; CV a = 23.12%; CV b = 8.49%; CV c = 11.33%)

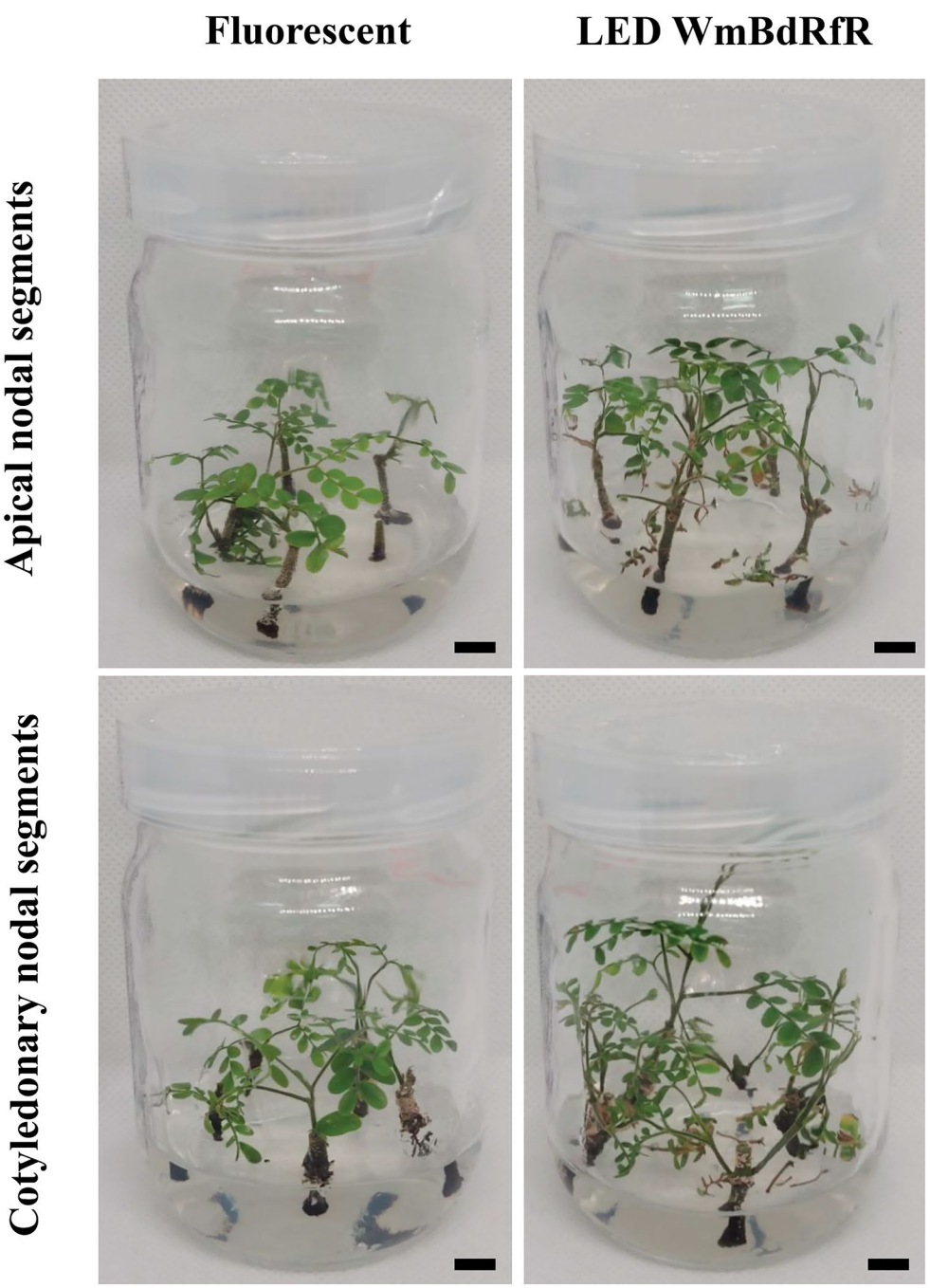


Fig. 5 Morphological aspects of *Paubrasilia echinata* shoots from apical and cotyledonary nodal segments after 120 days of in vitro incubation under fluorescent and LED W/mB/dR/fR lamps. Bars = 1 cm

identified, 17 of which were DAPs, including 1 unique in shoots from cotyledonary nodal segments and 2 unique to shoots from apical nodal segments (AFLU) (Fig. 7g and h).

In shoots from cotyledonary nodal segments cultured under LED W/mB/dR/fR lamp with fluorescent lamp (CLED/CFLU comparison) the KEGG pathway enrichment analysis revealed that the DAPs and unique proteins were associated with several biological pathways, including photosynthesis, metabolic pathways, glycolysis/gluconeogenesis, biosynthesis of secondary metabolites, ascorbate and aldarate metabolism, fatty acid degradation, and pentose phosphate pathway (Fig. 8a).

Shoots from apical nodal segments incubated under LED W/mB/dR/fR lamp compared to fluorescent lamp (ALED/AFLU comparison), the KEGG pathway enrichment analysis revealed that the DAPs and unique proteins were associated with several biological pathways, including carbon fixation in photosynthetic organisms, carbon metabolism, metabolic pathway, biosynthesis of amino acids, biosynthesis of secondary metabolites, and glycolysis/gluconeogenesis (Fig. 8b).

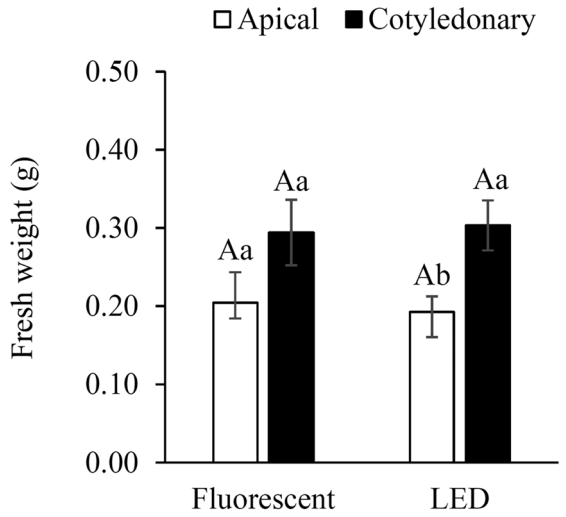


Fig. 6 Fresh weight (**g**) of *Paubrasilia echinata* shoots from apical and cotyledonary nodal segments after 120 days of in vitro incubation under LED W/mB/dR/fR and fluorescent lamps. Means followed by different letters show significant differences at the 5% probability level according to Tukey's test. Capital letters indicate significant differences for each type of explant (apical or cotyledonary nodal segments) among the different lamps. Lowercase letters indicate significant differences between the two types of explants (apical and cotyledonary nodal segments) for the same lamp. (mean ± standard deviation; $n = 8$; coefficient of variation = 28.7%)

Comparing the shoots from different explant types grown under the same lamp, only one KEGG pathway was significantly enriched in the CLED/ALED comparison, corresponding to the biosynthesis of amino acids (Fig. 8c). In contrast, no KEGG pathways were enriched in the CFLU/AFLU comparison.

In shoots from cotyledonary nodal segments under LED W/mB/dR/fR lamp compared to fluorescent (CLED/CFLU comparison) the DAPs proteins showed a protein-protein interaction network with photosynthesis, biosynthesis of secondary metabolites and ascorbate and aldarate metabolism KEGG pathways enrichment analysis (Fig. S1). The down-accumulated photosystem I reaction center subunit II, chloroplastic-like (paubrasilia_04543) and photosystem I reaction center subunit IV B, chloroplastic-like (paubrasilia_28831) proteins; the chlorophyll a-b binding protein of LHCII type 1-like (paubrasilia_25876) - the unique in shoots from CFLU and the up-accumulated oxygen-evolving enhancer protein 1, chloroplastic (paubrasilia_09643), formed an interaction network related to photosynthesis. Moreover, the proteins down-accumulated glucose-6-phosphate isomerase cytosolic isoform X1 (paubrasilia_34648) and transketolase, chloroplastic (paubrasilia_03703) interact in the biosynthesis of secondary metabolites network. In addition, the down-accumulated aldehyde dehydrogenase

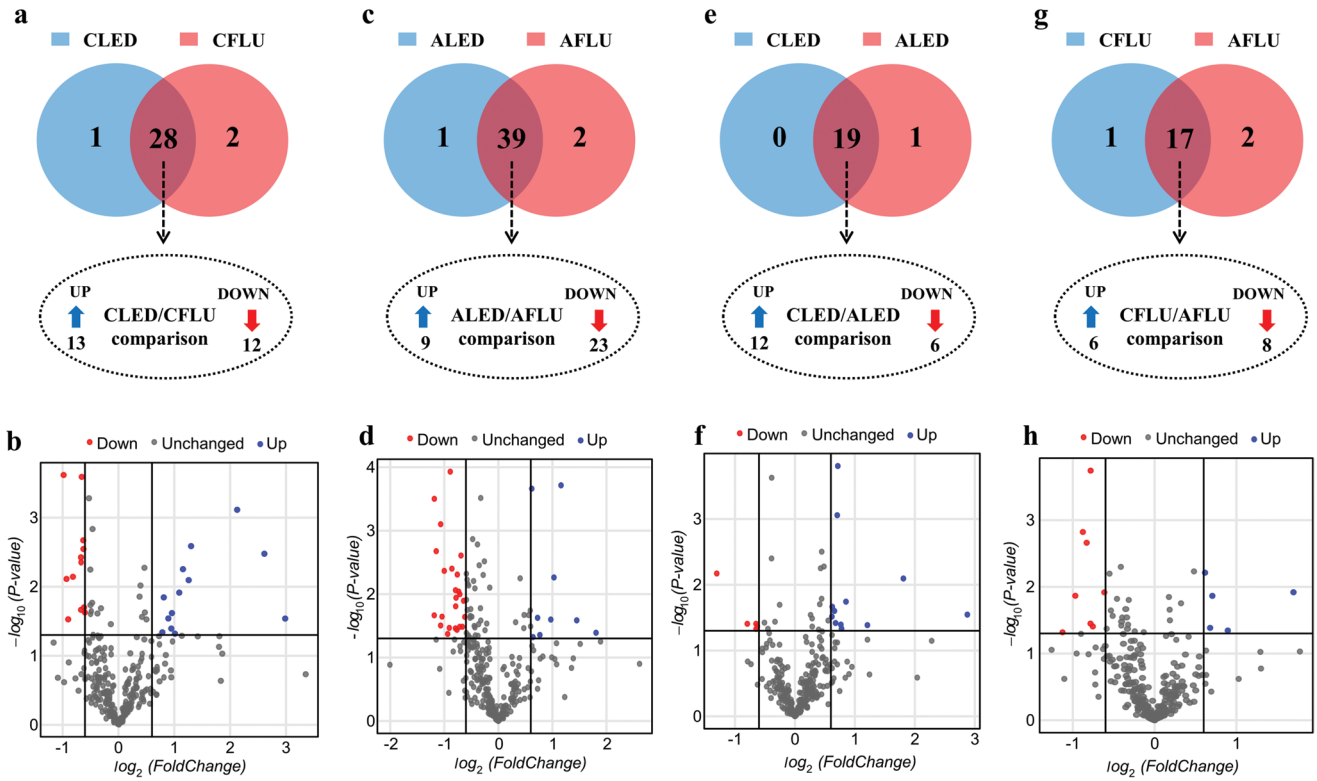


Fig. 7 Venn diagram and volcano plot of differentially accumulated proteins identified in enn diag*Paubrasilia echinata* shoots from cotyledonary [C] and apical [A] nodal segments at 120 days of incubation under LED and fluorescent (FLU) lamp, in the comparisons of CLED/CFLU (**a** and **b**), ALED/AFLU (**c** and **d**), CLED/ALED (**e** and **f**), and CFLU/AFLU (**g** and **h**)

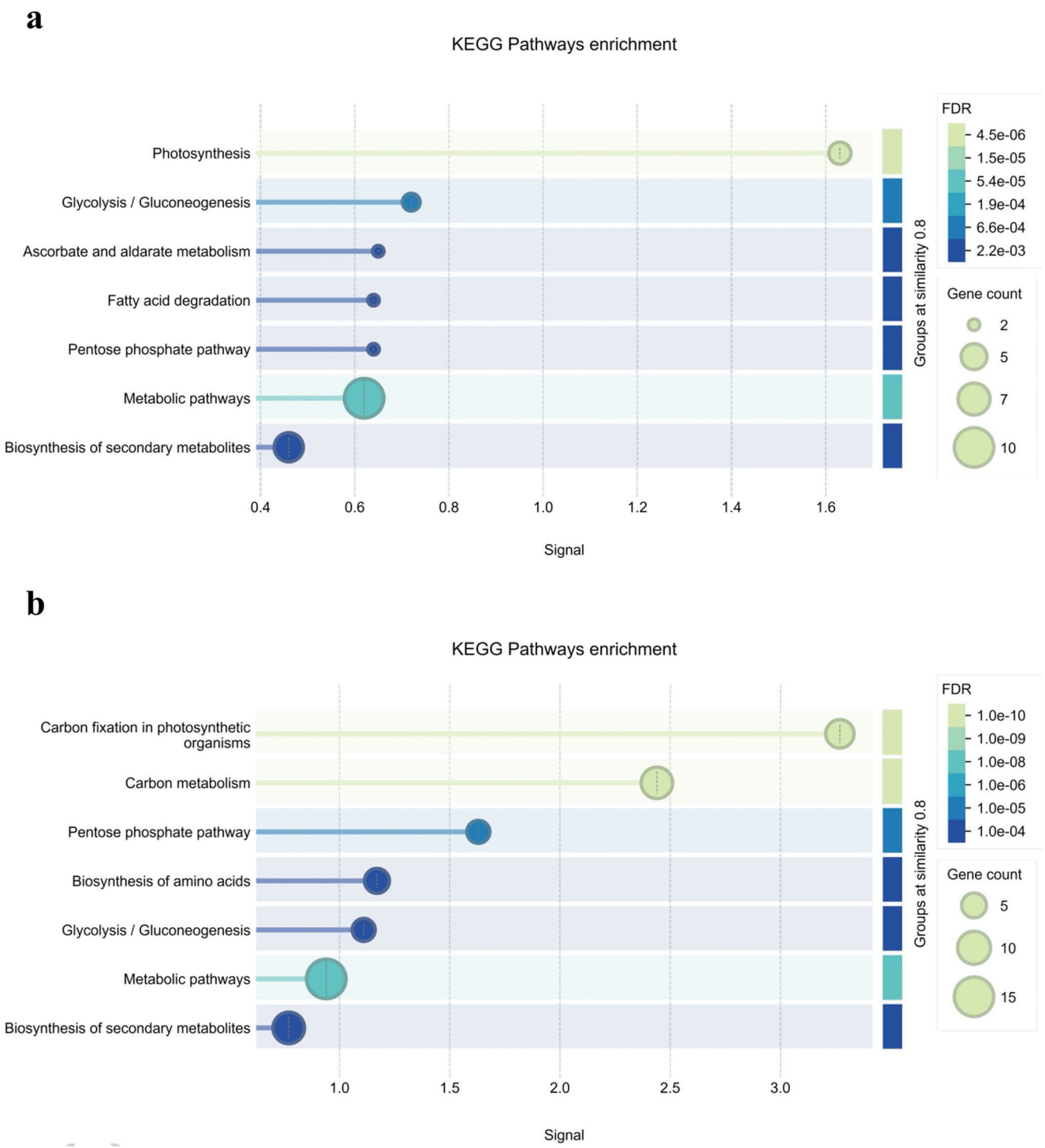


Fig. 8 KEGG pathway enrichment analysis for *Paubrasilia echinata* shoots from cotyledonary [C] and apical [A] nodal segments at 120 days of incubation under LED and fluorescent (FLU) lamps comparing CLED/CFLU (**a**), ALED/AFLU (**b**) and CLED/ALED (**c**). The comparison of shoots from cotyledonary under fluorescent lamp compared to apical (CFLU/AFLU) no pathway enrichment was observed

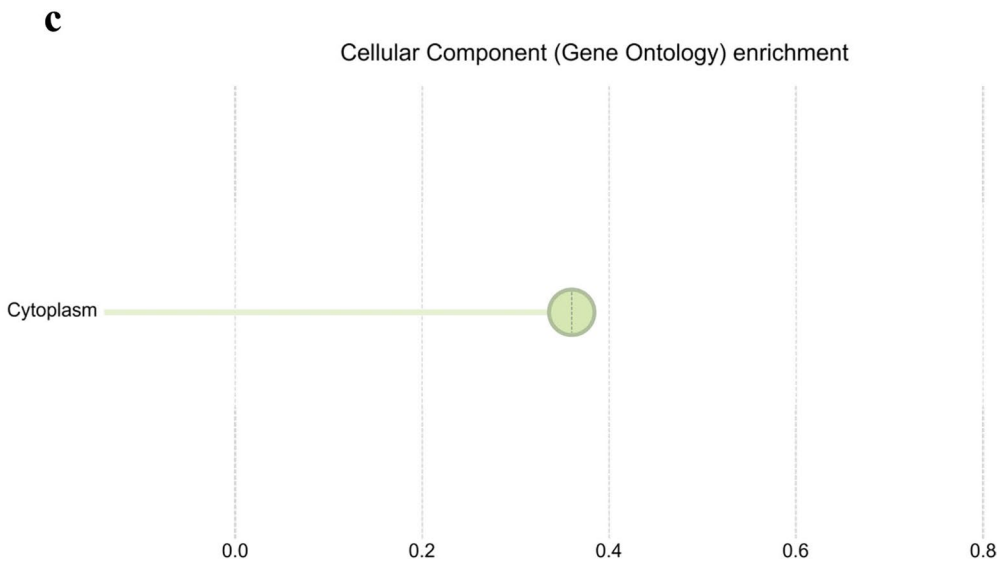


Fig. 8 (continued)

family 7 member A1 (paubrasilia_23050) showed an interaction with the up-accumulated protein alcohol dehydrogenase 1 (paubrasilia_51728). Shoots from apical nodal segments under LED W/mB/dR/fR compared to fluorescent lamp (ALED/AFLU) shower a protein-protein interaction network of several down-accumulated proteins related with biosynthesis of amino acids and secondary metabolites, carbon metabolism, and glycolysis/gluconeogenesis (Fig. S2). Moreover, a protein-protein interaction network of proteins in shoots from cotyledonary nodal segments, which promoted the best elongation under LED (CLED) compared to apical nodal (ALED) interacted with biosynthesis of amino acids (Fig. S3). Besides no KEGG pathways enriched comparing shoots from different type of explants under fluorescent lamp (CFLU/AFLU comparison), some proteins up-accumulated showed interaction, such as Ei-4; cathepsin B-like protease 2, actin-1-like and elongation factor 2-like isoform X1 (Fig. S4). Some proteins involved in growth and developmental processes specially were highlighted and further discussed.

Effects of explant type and spectra on photosynthetic pigments in shoots

The analysis of photosynthetic pigments revealed significant effects of explant type and light conditions (Fig. 9). Shoots from cotyledonary nodal segments exhibited higher contents of chlorophyll a (Fig. 9a), chlorophyll b (Fig. 9b), total chlorophyll (Fig. 9c) and carotenoids (Fig. 9d) when cultured under LED W/mB/dR/fR compared to fluorescent lamp, while those from apical nodal segments did not showed significant differences. As cotyledonary nodal segments allowed the development of shoots with greater elongation and higher fresh matter accumulation, as well as exhibited significantly higher levels of chlorophylls and carotenoids, they were selected for the subsequent PAs and hormonal profile analyses.

Effect of the light spectrum on the plant hormones contents in shoots from cotyledonary nodal segments

The LED W/mB/dR/fR lamp induced a higher content of BA (Fig. 10a), ACC (Fig. 10b) and ABA (Fig. 10c) in shoots from cotyledonary nodal segments compared with those grown under fluorescent lamp. In contrast, the highest SA content was observed in shoots incubated under fluorescent lamp (Fig. 10d). No significant differences were found in IBA (Fig. 10e), IAA (Fig. 10f), JA (Fig. 10g), and GA_3 (Fig. 10h) contents in shoots comparing the two lamps.

Effect of the light spectrum on the content of free PAs in shoots from cotyledonary nodal segments

A significantly higher content of total free PAs (Fig. 11a) and free Put (Fig. 11b) were observed in shoots from cotyledonary nodal segments grown under LED W/mB/dR/fR lamp compared with fluorescent. In contrast, no statistically significant differences were detected in Spd (Fig. 11c) and Spm (Fig. 11d) contents comparing the lamps.

Effect of explant type and IBA on ex vitro rooting

The type of explant and IBA concentrations did not affected significantly the rooting (%) of shoots (Fig. 12a), showing plantlets with similar rooting morphology comparing

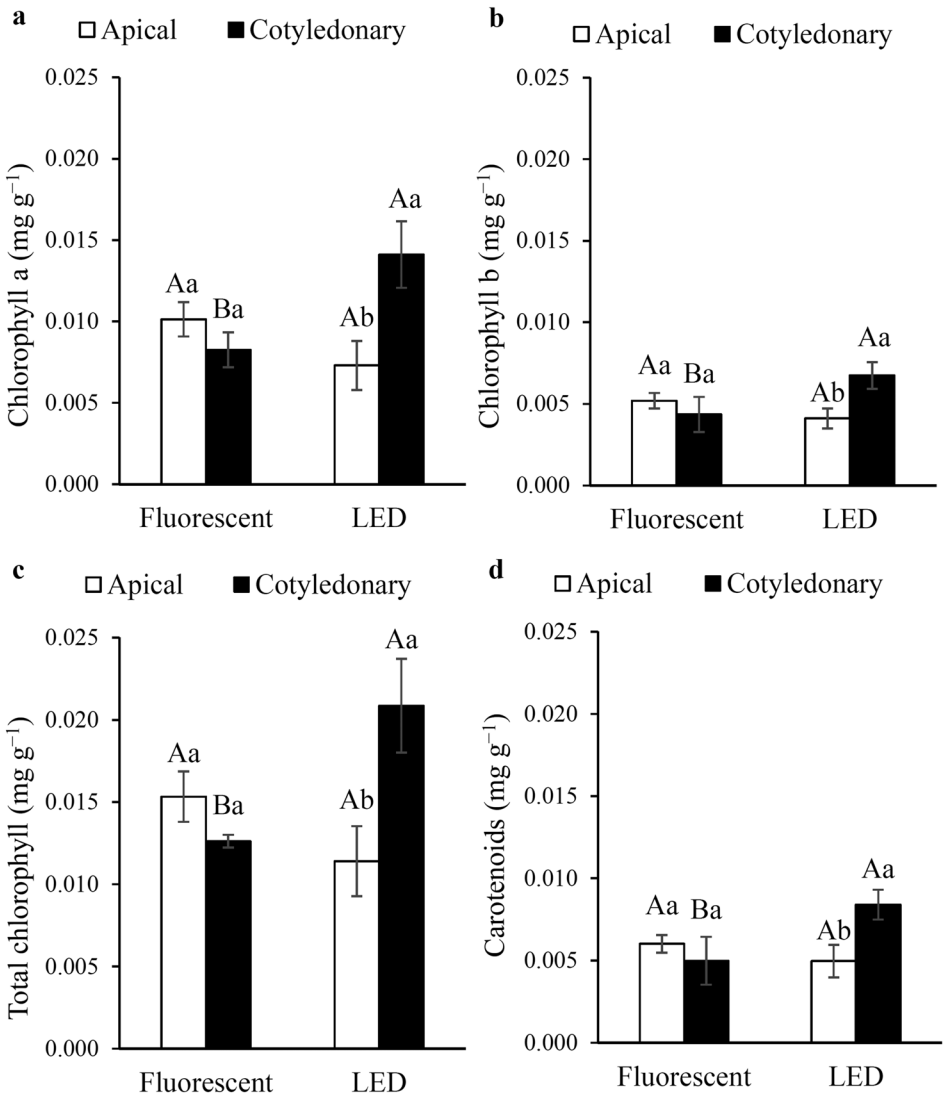


Fig. 9 Chlorophyll a (**a**), b (**b**), total chlorophyll (**c**) and carotenoid (**d**) content in *Paubrasilia echinata* shoots at 120 days of in vitro incubation under fluorescent and LED lamps. Means followed by the different letter indicate significant differences at the 5% probability level according to Tukey's test. Capital letters indicate significant differences for each type of explant (apical or cotyledonary nodal segments) comparing the different lamps. Lowercase letters indicate significant differences between two types of explants (apical and cotyledonary nodal segments) for the same lamp (LED or fluorescent). CV = coefficient of variation (mean ± standard deviation; $n = 3$; CV a = 25.7%; CV b = 20.3%; CV c = 23.8%; CV d = 21.9%)

shoots from apical (Fig. 12b) and cotyledonary (Fig. 12c) nodal segments. After 210 days on greenhouse, the plantlets showed 100% of survival (data not showed; Fig. 12d). Rooted plants (Fig. 12e) were transferred to the field (Fig. 12f), at the Instituto Biasse Socioambiental in Itaocara – RJ – Brazil. The Fig. 13 summarizes the entire process for plants obtention using in vitro culture and ex vitro rooting of shoots in *P. echinata.*

Discussion

In vitro morphogenesis of *P. echinata* is markedly influenced both the type of explant and the light spectrum, affecting shoot elongation and biomass increase by modulation of differentially accumulated proteins, plant hormones and PAs. Studies carried out with woody species have demonstrated that the position of the explant on the seedlings, such as cotyledonary and apical nodal segments, affects shoot induction and development (Aragão et al. 2017, 2017; Lerin et al. 2019; Souza et al. 2020a; Pessanha et al. 2022). Among the explant types, cotyledonary nodal segments have consistently promoted shoots with greater elongation, as observed in *C. legalis* (Aragão et al. 2017; Lerin et al. 2019), *C. fissilis* (Aragão et al. 2016), *D. nigra* (Pessanha et al. 2022) and also in *P. echinata* in the present study. The enhanced shoot elongation observed from cotyledonary nodal explants can be associated to a differential accumulation of endogenous levels of growth-promoting hormones such as auxins, cytokinins, gibberellins, and PAs, which are essential for cell division, elongation, and microtubule organization. On the other hand, apical nodal segments may be more developmentally mature and subject to apical dominance, with

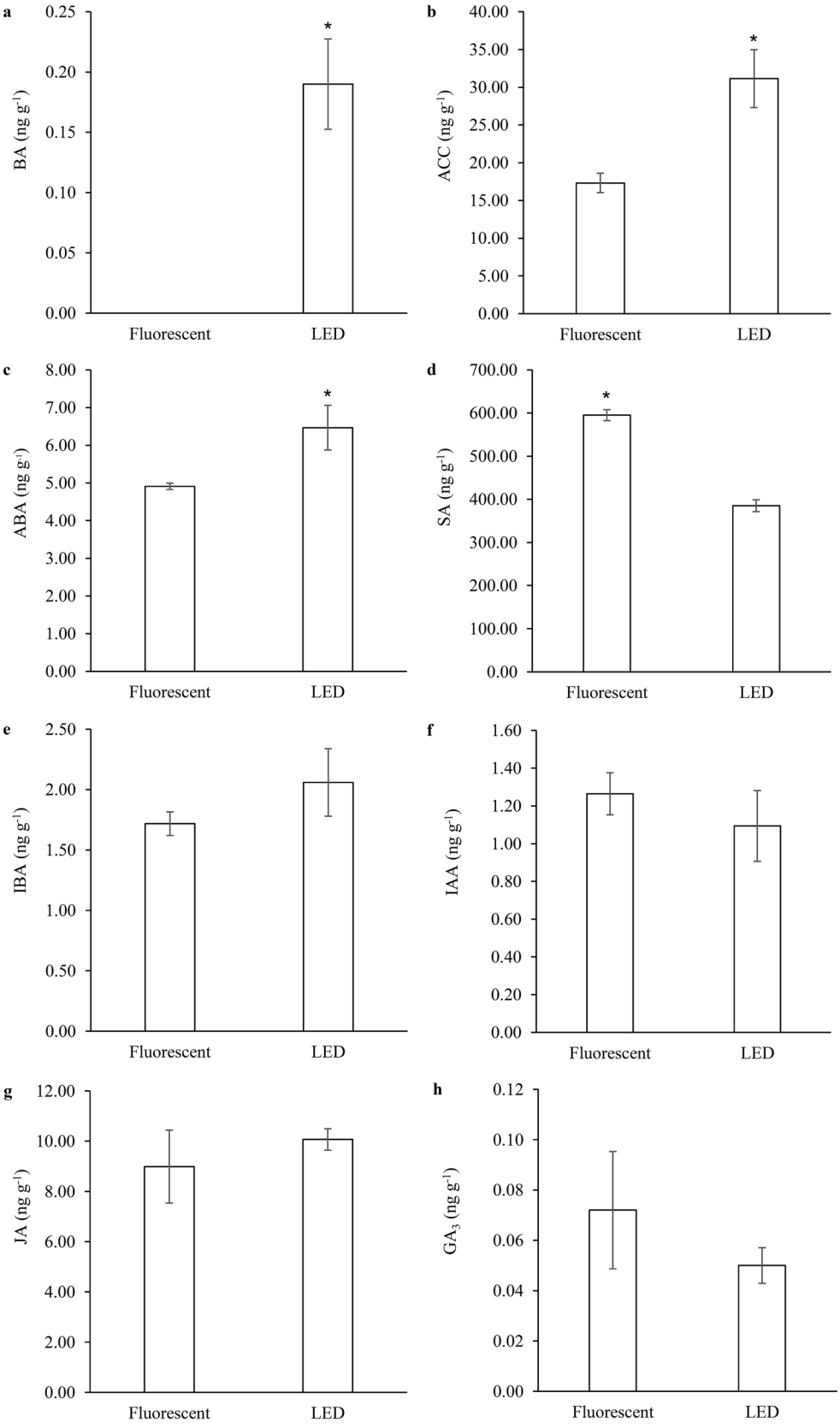


Fig. 10 Endogenous content (ng g^{-1} FW) of BA (**a**), ACC (**b**), ABA (**c**), SA (**d**), IBA (**e**), IAA (**f**),JA (**g**) and GA_3 (**h**) in shoots from cotyledonary nodal segments of *Paubrasilia echinata* at 120 days of in vitro incubation under LED W/mB/dR/fR and fluorescent lamps. Asterisks (*) indicates significant differences between the type of lamp at the 5% probability level according to Student's *t*-test. FW = fresh weight. CV = coefficient of variation. (mean ± standard deviation; $n = 5$; CV a = 0.10%; CV b = 24.2%; CV c = 5.7%; CV d = 49.1%; CV e = 1.9%; CV f = 1.2%; CV g = 9.5%; CV h = 0.06%)

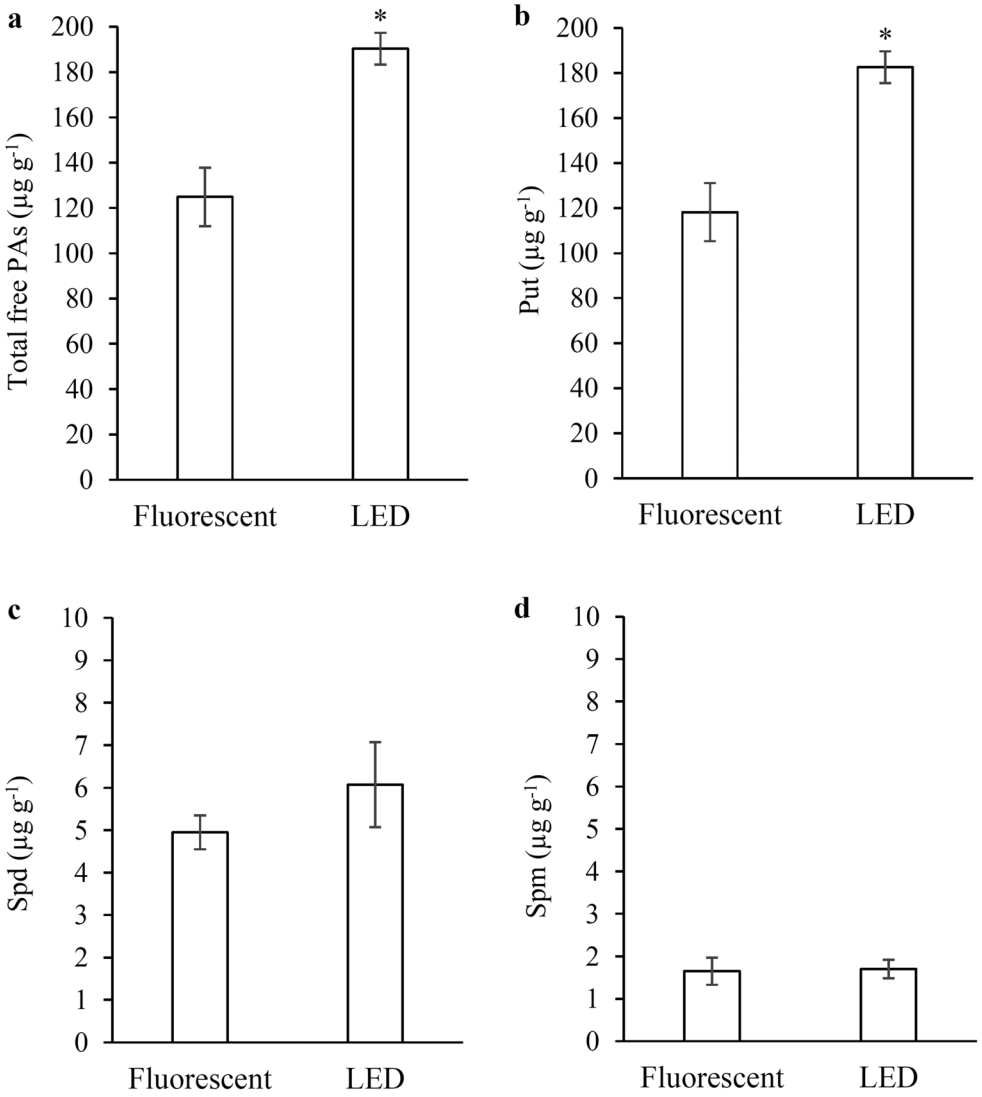


Fig. 11 Total free PAs (**a**), Put (**b**), Spd (**c**) and Spm (**d**) content (µg g^{-1} FW) in *Paubrasilia echinata* shoots from cotyledonary nodal segments at 120 days of in vitro incubation under LED and fluorescent lamp. Asterisks (*) indicates significant differences at the 5% probability level according to Student's *t*-test. FW = fresh weight. CV = coefficient of variation. (mean ± standard deviation; *n* = 3; CV a = 7.7%; CV b = 6.9%; CV c = 25.32%; CV d = 33.6%)

higher levels of auxin transport from the shoot apex that can restrict lateral shoot elongation, as observed in *P. echinata.*

Cytokinin is essential for in vitro shoot development from axillary buds in nodal segments in several species, such as *C. fissilis* (Nunes et al. 2002), *D. nigra* (Ivanova et al. 2006; Aragão et al. 2016; Hernández-García et al. 2021; Pessanha et al. 2022), *Actinidia chinensis* (Saeiahagh et al. 2019), *Actinia deliciosa* (Arruda et al. 2019), *Betula oycoviensis* (Vítamvas et al. 2020), *Myrcianthes pungens* (Souza et al. 2020a, b), *Campomanesia phaea* (Demétrio et al. 2021) e *Pinus koraiensis* (Liang et al. 2022). However, in *P. echinata*, the addition of cytokinin BA did not differ significantly from the control treatment. Instead, light quality provided by LED lamp had a predominant effect on shoot elongation. These results are consistent with findings in *C. legalis*, where BA alone did not improve shoot elongation, indicating that specific light spectra are required to enhance morphogenesis (Aragão et al. 2017; Lerin et al. 2019).

Blue and red wavelengths are crucial in regulating plant development, as their photoreceptors modulate key morphogenetic processes (He et al. 2020; Silva et al. 2020). LED sources combining red and blue wavelengths have promoted shoot development in various species, such as *Camellia oleifera* (He et al. 2020), hybrids of *Corymbia* spp (Souza et al. 2020a), *Swertia chirata* (Gupta and Karmakar 2017), *Prunus cerasus* and *P. canescens* (Sarropoulou et al. 2023). Blue light is mainly perceived by phototropins and cryptochromes. Phototropins regulate phototropism, chloroplast movement and stomatal opening (Briggs and Christie 2002), while cryptochromes participate in flowering, inhibition of etiolation, stomatal regulation, and root elongation (Canamero et al. 2006; Bach et al. 2018). Red (R) and far-red (fR), in turn, are detected by phytochromes, which control seed germination, stomatal development, flowering transition, leaf movement, senescence, and shade avoidance responses (Franklin and Quail 2009). In *C. fissilis* (Oliveira

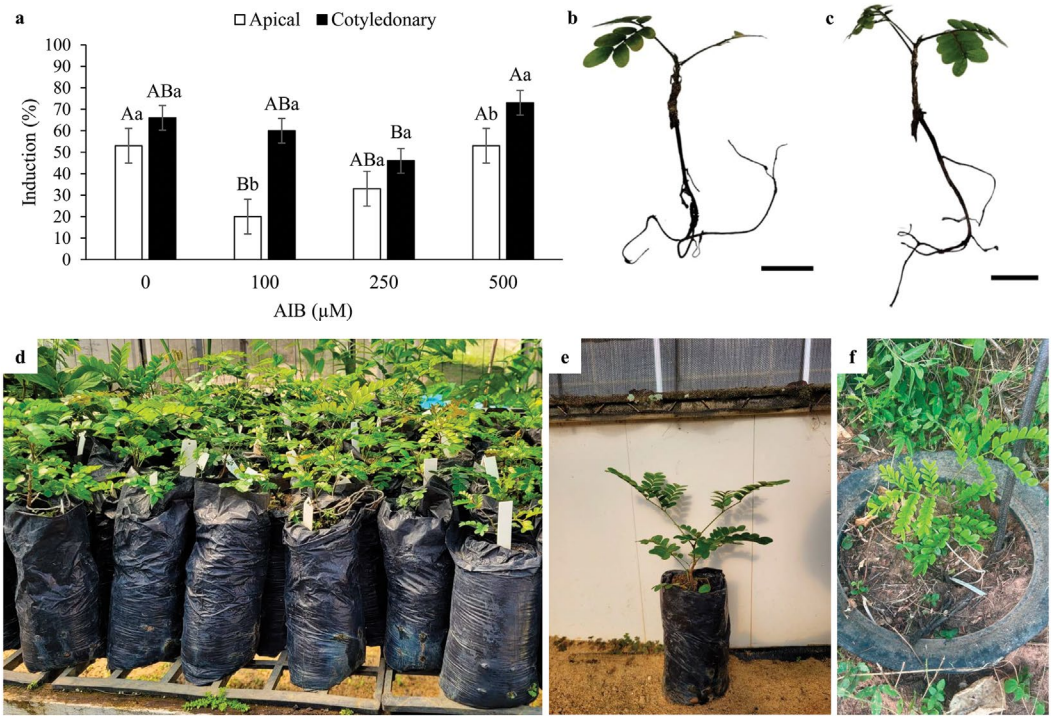


Fig. 12 Ex vitro root induction of shoots from apical and cotyledonary nodal segments under different indole-3-acetic acid (IBA) concentrations (**a**), showing rooted plantlets of shoots from apical (**b**) and cotyledonary nodal segments, following transference to greenhouse (**d** and **e**) and to the field (**f**) at Instituto Biasse Socioambiental – Itaocara, RJ, Brazil. Coefficient of variation a = 19.6%. Bars a,b = 1 cm

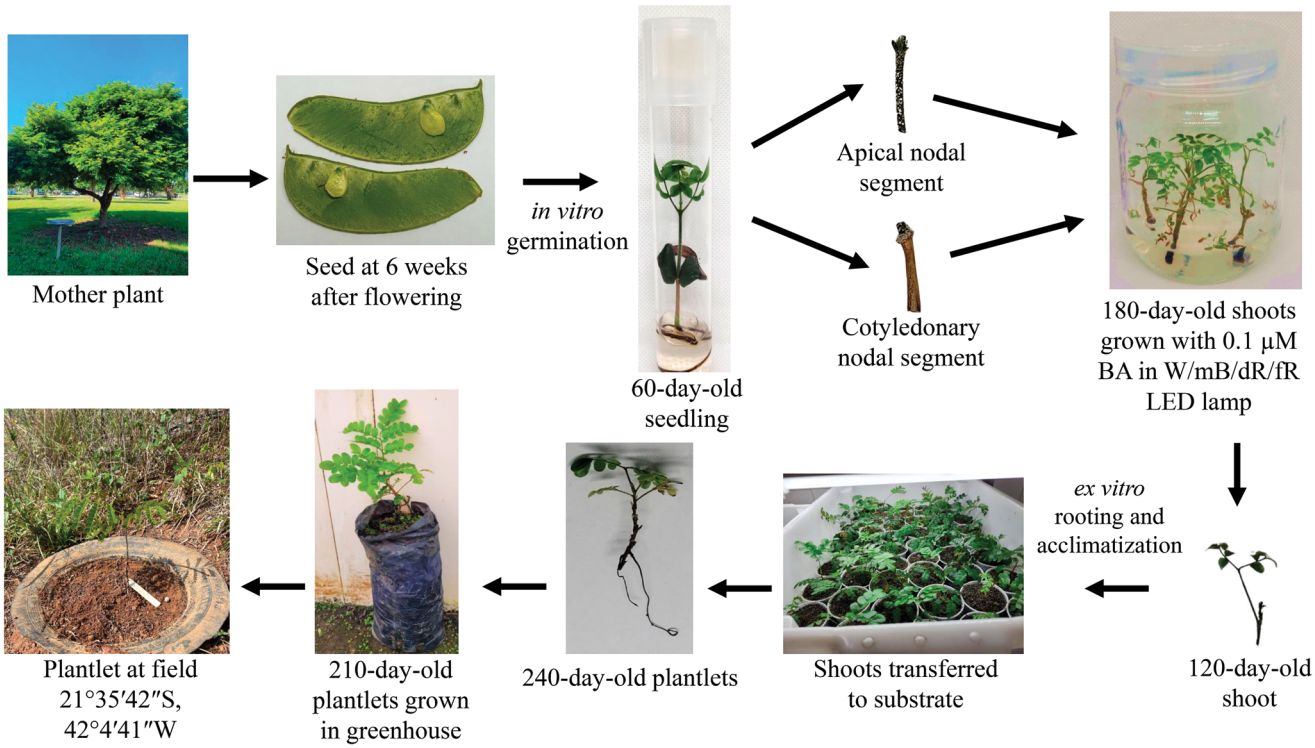


Fig. 13 Scheme showing the entire process of in vitro propagation of *Paubrasilia echinata*, including ex vitro rooting and acclimatization, and transference into the field

et al. 2020) and *Gallesia integrifolia* (Rodrigues 2023) the LED W/mB/dR lamp enhanced shoot growth, reinforcing the importance of red-enriched spectra for in vitro morphogenesis. In *C. legalis*, LED lamps emitting W/lB/dR/fR resulted in greater elongation of shoots from cotyledonary nodal segments compared to fluorescent lamp (Lerin et al. 2019). Similarly, in *P. echinata*, the LED lamp with W/mB/dR/fR significantly increased shoot length in both cotyledonary and apical nodal explants, suggesting a key role of R and fR light in shoot elongation. These findings suggest that the combination of low blue and red lights induces a shade-avoidance response, enhancing cell elongation and stem extension even in the absence of fR light. In *P. echinata*, shoot elongation was more strongly influenced by light quality than by the addition of BA to the culture medium, highlighting the importance of optimizing light spectra to improve in vitro propagation of woody species.

In addition, shoots of *P. echinata* grown under LED W/mB/dR/fR lamp showed endogenous BA, which was not detected in shoots cultivated under fluorescent lamp. In *Malus sylvestris*, BA is absorbed in the shoot region but remains concentrated in the basal part of the nodal segment, promoting bud development (Nordström and Eliasson 1986). In *Ulmus campestris*, BA is rapidly absorbed (within 30 min), and after 6 h it is degraded into adenine through side-chain cleavage (Biondi et al. 1984). In *Arabidopsis thaliana*, high light intensity reduced cytokinin levels, resulting in increased photooxidative stress and decreased photosynthetic activity (Cortleven et al. 2014). Thus, the lower BA contents in *P. echinata* shoots under fluorescent lamp may be associated with the higher luminous flux of this lamp compared to LEDs. LED lamps (4.5 W) emit less heat, consume less energy, and have a lower luminous flux (160 lm), while fluorescent lamps (15 W) provide 316 lm (Santos et al. 2015). Similarly, in *Triticum aestivum*, a low deep R/fR ratio was shown to reduce cytokinin content (Lei et al. 2022). Therefore, the reduced BA concentration in *P. echinata* shoots under fluorescent lamp may be due to degradation induced by the higher luminous flux. To further understand how light quality regulates in vitro morphogenesis, proteomic approaches are essential for identifying the proteins and metabolic pathways modulated under these conditions.

The light spectrum and explant type also induced differential protein accumulation during shoot development in *P. echinata* (Supplementary Table S1). Among the DAPs, shoots from cotyledonary nodal segments grown under the LED W/mB/dR/fR lamp showed exclusive accumulation of 70 kDa heat shock protein (HSP; paubrasilia_14874), which was not detected in shoots grown under fluorescent lamp (CLED/CFLU comparison). A similar response was reported in *C. fissilis*, where higher accumulation of HSPs were reported in shoots grown under LED W/mB/dR lamp compared with those from fluorescent lamp (Oliveira et al. 2020). HSP is a conserved family of molecular chaperones that play a central role in plant responses to abiotic and biotic stresses. They prevent protein misfolding, reduce aggregation of denatured proteins, and maintain proteome stability, thereby ensuring cellular homeostasis (Fang et al. 2021; Yin et al. 2023). Alteration in HSP expression, either suppression or overaccumulation, can impair plant growth and development, as reported in Arabidopsis (Sung and Guy 2003; Jungkunz et al. 2011). Therefore, the increased abundance of HSPs in shoots grown under the LED W/mB/dR/fR lamp may be associated with enhanced cellular protection and protein homeostasis, contributing to the greater shoot elongation observed in *P. echinata* under this light condition.

In addition, the proteins ascorbate peroxidase (APX) and peroxidases (PODs) are key enzymes in the plant antioxidant system, acting synergistically to maintain redox homeostasis and protect cells against oxidative damage. APX catalyzes the reduction of hydrogen peroxide (H_2O_2) to water using ascorbate as an electron donor, preventing oxidative damage to proteins, lipids, and cellular membranes (Corpas et al. 2024; Yoshimura and Ishikawa 2024). In tobacco, overexpression of the *StAPX* gene significantly reduced H_2O_2 accumulation, strengthening the antioxidant defense system (Sun et al. 2013). PODs contribute to H_2O_2 detoxification and participate in lignin biosynthesis and cell wall formation, processes crucial for plant growth and structural integrity (Castillo 2025). In *Phoenix dactylifera*, shoots grown under LED lamp (18 red:2 blue) exhibited enhanced growth, higher multiplication rates, and increased peroxidase activity compared with those grown under fluorescent lamp (Al-Mayahi 2016). In the present study, shoots derived from cotyledonary nodal segments of *P. echinata* grown under LED W/mB/dR/fR spectrum showed increased accumulation of L-ascorbate peroxidase 2 (paubrasilia_31781) and peroxidase A2-like (paubrasilia_52999) compared to those grown under fluorescent lamp (CLED/CFLU comparison). Similarly, shoots from apical nodal segments exhibited higher levels of peroxidase 73 (paubrasilia_25491) and peroxidase 51-like (paubrasilia_25244) under the same LED spectrum compared to those from fluorescent (ALED/AFLU comparison). Thus, the increased abundance of these antioxidant enzymes under LED conditions suggests a more efficient ROS-scavenging system and improved redox regulation, which may have favored cell expansion and contributed to the increased shoot elongation observed in shoots derived from cotyledonary and apical nodal segments under LED WmBdRfR lamp.

Zeaxanthin epoxidase (ZEP) catalyzes the epoxidation of the carotenoid zeaxanthin to violaxanthin in the xanthophyll

cycle, providing essential precursors for abscisic acid (ABA) biosynthesis (Wu et al. 2023; Yadav et al. 2023). Thus, ZEP plays a pivotal role in linking photoprotective mechanisms to hormonal regulation under stress and developmental conditions (Premachandran 2022). In *C. fissilis*, the reduction in shoot length during successive subcultures was associated with a decrease in ABA levels (Lerin et al. 2021). In our study, the higher accumulation of ZEP (paubrasilia_47510) and carotenoids in shoots from cotyledonary nodal segments grown under LED lamp may induced the increase in ABA levels, which could contribute to the shoot developmental performance in this light condition compared to fluorescent lamp. Moreover, elevated ABA levels are known not only to regulate stress tolerance and developmental processes but also to modulate other hormonal networks. For instance, ABA can induce the expression of ACC synthase and ACC oxidase, enhancing ethylene biosynthesis in several plant species under stress or developmental transitions, as well as ABA-treated tissues show higher ethylene emission (Hansen and Grossmann 2000; Sharp 2002; Zhang et al. 2009). Conversely, ABA often acts antagonistically to SA, suppressing SA biosynthesis genes (ICS1, NPR1), reducing SA accumulation and SA-dependent defense (Yasuda et al. 2008; Moeder et al. 2010; Alazem et al. 2019). Therefore, the higher ZEP–ABA response observed in cotyledonary-derived shoots may contribute indirectly to hormonal reprogramming, favoring ethylene-associated growth responses while down-regulating SA-mediated pathways in *P. echinata*. This hormonal balance could support enhanced shoot elongation and morphogenetic performance under LED light conditions. In addition, the higher SA content in shoots grown under the fluorescent lamp may have associated with the greater heat emission and higher luminous flux of this lamp compared with LED. These conditions likely induced stress and negatively affected the growth of *P. echinata* shoots. SA is a key plant hormone involved in responses to stress, acting in plant defense and growth (Seyfferth and Tsuda 2014; Ahmad et al. 2019; Elsisi et al. 2024). Studies emphasize the importance of SA in enhancing plant resistance to both biotic and abiotic stresses (Benjamin et al. 2022; Khan et al. 2022). In *C. fissilis*, a significant increase in endogenous SA content in shoots during the fourth subculture compared with the first, was associated with reduced shoot growth and lower rooting potential (Oliveira et al. 2022). This effect may be explained by the antagonistic relationship between SA and auxin, as SA is known to reduce auxin levels, a hormone required for root induction (De Klerk et al. 2011).

The Oxygen-Evolving Enhancer Protein 1 (OEE1) is an essential extrinsic subunit of photosystem II (PSII), also referred to as the PsbO protein (Carius et al. 2019). This protein plays a fundamental role in the light-driven water-splitting reaction, contributing to the release of oxygen and electrons during photosynthesis (Deng et al. 2025). In our study, we observed that shoots derived from cotyledonary nodal segments and grown under a white-blue-red-far red (WmBdRfR) LED lamp exhibited increased accumulation of OEE1 (paubrasilia_09643), along with higher levels of photosynthetic pigments, including chlorophylls a and b and carotenoids. This suggests that the enhanced presence of OEE1 under LED lighting may contribute to improved photosynthetic efficiency in these shoots. The increase in photosynthetic pigments under LED lamp can be attributed to the specific light spectrum provided by the LEDs. Red and blue light wavelengths are efficiently absorbed by chlorophylls and carotenoids, leading to enhanced light absorption and energy capture, which are vital for photosynthesis (Liu and Van Iersel 2021). Additionally, the presence of OEE1 stabilizes the PSII complex, facilitating efficient electron transport and oxygen evolution, thereby supporting overall photosynthetic activity (Mayfield et al. 1987). Thus, the upregulation of OEE1 and the increased accumulation of photosynthetic pigments in shoots grown under LED lamp indicate a synergistic enhancement of photosynthetic efficiency. This combination likely contributes to the observed improved shoot growth under LED conditions.

The Tubulin beta chain (paubrasilia_16187) was up-accumulated in shoots derived from cotyledonary segments compared to apical nodal segments grown under the LED W/mB/dR/fR lamp, (CLED/ALED comparison). Tubulin, as the main structural component of microtubules, plays a central role in determining the orientation of cell division and expansion, thereby directly influencing morphogenesis and plant development (Hamada 2014; Hsiao and Huang 2023). PAs are known to modulate tubulin polymerization and self-association, acting as key regulators of microtubule dynamics (Mechulam et al. 2009). In *C. legalis*, shoots grown under a LED W/lB/dR lamp exhibited higher levels of free Put and increased tubulin accumulation compared with those under fluorescent lamp (Lerin et al. 2019). The greater abundance of tubulin in shoots derived from cotyledonary nodal segments, together with their higher endogenous content of total free Put suggests a more active microtubule organization, which may contribute to the enhanced shoot development observed in these shoots. These observations are consistent with additional literature reporting that microtubule stability and orientation are tightly regulated by cytoskeletal-associated proteins and signaling molecules, which influence cell elongation, division plane determination, and organ morphogenesis (Mathur and Hülskamp 2002; Nick 2013). Together, these findings suggest that the greater abundance of tubulin in cotyledonary nodal segments, in combination with elevated levels of free Put reflects a more active microtubule network. This enhanced

cytoskeletal organization likely contributes to the superior shoot elongation and morphogenetic performance observed under LED W/mB/dR/fR compared with fluorescent lamp and apical nodal segments.

Moreover, Put is associated with cell cycle progression (G1/S to G2/M) and division activity (Weiger and Hermann 2014), and the higher Put levels in *P. echinata* shoots under LED W/mB/dR/fR lamp enriched in red and blue shoots may stimulate cell proliferation and elongation. In *C. fissilis*, light spectra from LED lamp W/mB/dR increased the endogenous Put content and altered the accumulation profile of proteins related to metabolism, stress responses, biosynthesis, protein modification, and light stimulus, promoting shoot elongation (Oliveira et al. 2020). Similarly, in *G. integrifolia*, where this LED spectrum also increased Put and total free PAs, as well as proteins related with Krebs cycle compared to light from fluorescent lamp (Rodrigues 2023). In *C. legalis*, LED spectra composed of W/lB/dR/fR or W/lB/dR promoted greater shoot elongation from cotyledonary nodal segments compared to fluorescent light, likely due to shade-avoidance responses induced by the combination of low blue and red wavelengths. Under W/lB/dR LED, these shoots also showed higher accumulation of Put and Spd, along with proteins involved in metabolic regulation, cellular organization, photosynthesis, and stress responses (Lerin et al. 2019).

The type of explant and IBA concentrations did not significantly affected ex vitro rooting (%) of shoots, which ranged from 50 to 65% for both explant types. Further studies are need to be performed aiming to improve ex vitro rooting in *P. echinata*. Although tree species often require an auxin, such as IBA, to promote adventitious rooting, our results indicate that the tested auxins were not effective in enhancing rooting in micropropagated shoots of *P. echinata*. In *C. fissilis*, shoots achieved high ex vitro rooting rates (over 90%), without the application of IBA (Ribeiro et al. 2022). The authors suggested that this ability is likely due to endogenous IAA metabolism and signaling, which can trigger root induction in these shoots. Ex vitro rooting has been successfully applied in other woody species, but often requires exogenous auxin to promote rooting, as observed for *Tecomella undulata* (Varshney and Anis 2012) and *Albizia lebbeck* (Perveen et al. 2013), both treated with 200 µM IBA for 30 min. Further investigations are necessary to optimize rooting induction in *P. echinata*.

Conclusions

The in vitro development of *P. echinata* is primarily influenced by explant type and light quality. Cotyledonary nodal segments were more efficient in promoting elongated shoots than apical nodal segments. The LED lamp composed of white, medium-blue, red, and far-red (W/mB/dR/fR) light spectra stimulated greater shoot elongation, biomass accumulation, and modulation of key proteins associated with photomorphogenesis, stress responses, hormonal regulation, and cellular organization. LED illumination also promoted higher endogenous accumulation of BA, Put, and proteins related to photosynthesis, antioxidant activity (APX and PODs), HSPs, and tubulin, suggesting enhanced cellular stability, redox homeostasis, cytoskeletal organization, and photosynthetic efficiency. Ex vitro rooting averaged 50–65%, with no significant effect of IBA or explant type, indicating that further adjustments are needed to optimize the rooting and acclimatization stages of *P. echinata*.

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Author contributions CSC conceived the study, designed the experiments, supervised all stages of the work, acquired funding, and contributed to manuscript writing and final revision. JOS, PRC, and LTMM were responsible for in vitro shoot culture, ex vitro rooting, and statistical analyses. RGV and YRSR performed the polyamine analyses. JOS, JNN, and RC-S provided support for the hormone analyses. JNN, MSR, and VS carried out the proteomic analyses. AB, JOS, and CSC were responsible for the field plantation of the first micropropagated plantlet. All authors contributed to manuscript writing and approved the final version.

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Data availability The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD070163. All identified proteins are available in the supplementary material.

Code availability PXD070163.

Declarations

Ethical approval Not applicable

Conflict of interest The authors of the manuscript have no competing interests to declare that are relevant to the content of this article.

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