In vitro culture of *Mouriri elliptica* (Mart.) under conditions that stimulate photoautotrophic behavior

Elisvane Silva de Assis[1], Aurélio Rubio Neto[1], Letícia Rigonato de Lima[1], Fabiano Guimarães Silva[1*], Márcio Rosa[2], Sebastião Carvalho Vasconcelos Filho[1], Mariluza Silva Leite[1]

[1]Federal Institute of Education, Science and Technology of Goiano, Rio Verde Campus, Rod, Sul Goiana Km 01, Cx. P. 66. CEP 75.901-970, Rio Verde –Goiás, Brazi
[2]University of Rio Verde – Uni RV, Rio Verde Campus, Fontes do Saber farm, Cx. P. 104. CEP: 75901-970, Rio Verde – Goiás, Brazil

*Corresponding author: fabianoifgoiano@gmail.com

Abstract

Micropropagation has been efficiently used to mass produce seedlings of species that are difficult to multiply via conventional methods. Thus, the present study aimed to analyze the *in vitro* culture of *Mouriri elliptica* (Mart.) seedlings under conditions that stimulate photoautotrophic behavior. The nodal segments were grown in 50% salt wood plant medium (WPM) in the presence and absence of sucrose and subjected to different light intensities (0, 50, 75, 100, and 150 µmol $m^{-2}s^{-1}$). The evaluations were performed after 60 days of culture and considered growth and morphoanatomic characteristics. There was an exponential increase in the number of shoots and leaves in the seedlings cultured in the absence of sucrose with increasing light intensity. Additionally, greater total and leaf dry weights were recorded in the seedlings cultured in sucrose-supplemented medium at a light intensity close to 100 µmol $m^{-2}s^{-1}$. Morphoanatomic changes were observed in the leaves at different light intensities, both in the presence and absence of sucrose. As the light intensity increased, the supplementation of the medium with sucrose became unnecessary. Thus, photoautotrophic conditions can be used for micropropagation of the species.

Keywords: autotrophic micropropagation; "croada"; light intensity; morphoanatomy; stomatal crypt; sucrose.
Abbreviations: Ab Ep T_abaxial epidermis thickness; Ad Ep T_adaxial epidermis thickness; ANOVA_analysis of variance; CP T_chlorophyll parenchyma thickness; St Cr Dn _ stomatal crypt density; St Cr Dp _stomatal crypt depth; St Cr O_stomatal crypt opening area; WPM_Wood Plant Medium.

Introduction

Mouriri elliptica (Mart.) belongs to the family Melastomataceae. It is a fruit tree that occurs naturally in several Brazilian states, being very common in the Goiás Cerrado biome, and it has been classified as a non-traditional tropical fruit (Rufino et al., 2010). It is popularly known as "croada", "croadinha", "coroa de frade", "puçá", "puçazeiro", or "manipuçá". When ripe, its fruit is sweet and rich in antioxidant compounds, such as vitamin C, anthocyanins, carotenoids and flavonoids; it can be eaten raw by humans or processed into jellies (Silva et al., 2001; Rufino et al., 2010; Rufino et al., 2011). The plant also has medicinal potential. The results of the application of *M. pusa* and *M. elliptica* leaf extracts in rodents indicate is an alternative treatment for acute ulcers, with these extracts exhibiting a gastroprotective effect and promoting healing. The extracts also have a protective effect against *Helicobacter pylori*, which is a microorganism that causes serious gastrointestinal diseases. These effects have been attributed to phenolic constituents in the form of flavonoids and tannins identified in the plants' leaves (Moleiro et al., 2009; Vasconcelos et al., 2010b). Leaf extracts from this species show no toxicity in treated animals, which is an important factor in its pharmacological applicability (Moleiro et al., 2009). There are currently no studies on "croada" micropropagation, despite the plant's various uses. Its seeds have a rigid coat, hindering its sexual

reproduction, as reported by Vasconcelos et al. (2010a), requiring the application of practices that promote overcoming dormancy. Therefore, propagation of this important species by seeds may not meet the demands for its seedlings. *In vitro* propagation provides a greater chance of producing seedlings that could be used for crops or reforestation. In conventional micropropagation, i.e., photomixotrophic micropropagation, the explants are cultured in flasks that restrict gas exchange; that have a high relative humidity, high ethylene concentration, low CO_2 concentration, low-density flow of photosynthetically active photons; and that use sucrose as the main metabolic energy source. This system may cause anatomical and physiological disorders in the seedlings, hindering the normal function of the photosynthetic apparatus (Xiao et al., 2011), as observed in the *in vitro* culture of *Billbergia zebrina* (Herbert), in which the supply of sucrose reduces the quantity of photosynthetic pigments (Martins et al., 2015). This is one of the features that may cause seedling losses during the acclimatization process, increasing production costs. Thus, photoautotrophic micropropagation has been investigated using a number of different practices, such as total or partial elimination of sucrose from the culture medium (Xiao and Kozai, 2006), enrichment of atmospheric CO_2 (Saldanha et al., 2013; Saldanha et al., 2014), reduction of the relative

humidity and ethylene concentration in the culture flask using seals that allow greater gas exchange (Saldanha et al., 2012), replacement of agar with alternative support materials such as Florialite® (Saldanha et al., 2014) or leaf litter and coconut fiber (Deb and Pongener, 2013), and increases in light intensity (Zhang et al., 2009; Sáez et al., 2012). These conditions can increase plant growth, improve physiological characteristics, and facilitate seedling acclimatization to *ex vitro* conditions by promoting the development of the photosynthetic apparatus (Walters, 2005; Santana et al., 2008; Iarema et al., 2012).

Anatomical and physiological evaluations and growth analysis can be performed to investigate autotrophic development, as observed in studies by Iarema et al. (2012), who evaluated the photoautotrophic propagation of *Pfaffia glomerata* (Spreng.); Sáez et al. (2012), who studied a culture of *Castanea sativa* Mill; Fan et al. (2013), who studied *Solanum lycopersicum* L.; and Dong et al. (2014), who studied an *in vitro* culture of *Triticum aestivum* L.

Thus, the present study aimed to analyze the behavior of *Mouriri elliptica* (Mat.) seedlings subjected to a culture medium without sucrose and with an increased light intensity in the environment by evaluating the growth and morphoanatomic characteristics.

Results and discussion

The increase in light intensity eliminated the requirement for M. elliptica (Mart.) seedlings for sucrose in the culture medium

There was an interaction between the light intensities (0, 50, 75, 100, and 150 µmol $m^{-2}s^{-1}$) and the sucrose levels that affected the seedling length, the number of shoots and leaves, the total dry weight and the leaf dry weight. An isolated effect of the factors on the leaf area and specific leaf area was observed ($p \leq 0.05$). Traditionally, during *in vitro* culturing, seedlings are kept in a growth room under low light intensity, and sucrose is used as the metabolic energy source for the explants (Zhang et al., 2009; Arigita et al., 2002). Fig 1 shows *Mouriri elliptica* (Mart.) seedling growth in photomixotrophic (A-E) and photoautotrophic systems (F-J).
A greater seedling length was observed in the absence of light and in the presence of sucrose (Figs 1 and 2), demonstrating etiolation characteristics that are most likely due to the seedlings' sensitivity to endogenous auxin (George, 1993), considering that sucrose availability in the culture medium induced the *M. elliptica* (Mart.) seedlings to metabolize auxin, a result previously observed in *Arabidopsis* (Sairanen et al., 2012).

Etiolation of these seedlings *in vitro* can be advantageous to the multiplication process, allowing their nodal segments to be used as explants, as observed in a study by Suzuki et al. (2004) and to obtain new shoots of crop species, as in pineapple plants (Moreira et al., 2003). However, etiolation is a characteristic related to the inefficiency of the photosynthetic apparatus (Solymosi and Schoefs, 2010) and susceptibility to photoinhibition (Long, 1994), which may compromise the acclimatization process. The maximum number of leaves (3.8 leaves per plant) was obtained in seedlings cultured in the medium with sucrose at a light intensity of 67 µmol $m^{-2}s^{-1}$ (Fig 3a). The *M. elliptica* Mart. seedlings remained less dependent on high light intensities when sucrose was supplemented in the culture medium, forming tissue even in the absence of light (Fig 1a). However, the highest accumulated total dry weight (40.36

mg) and leaf dry weight (26.67 mg) occurred when the seedlings were cultured at a light intensity of approximately 100 µmol $m^{-2}s^{-1}$ (Fig 3c and 3d). These results corroborate those of Zhang et al. (2009), who observed higher fresh and dry weights of *Momordica grosvenori* plants under increased environmental light intensity. The number of leaves and shoots increased linearly with an increasing light intensity in the seedlings cultured in the medium without sucrose (Figs 3a and 3b). There was no difference in these characteristics between the culture medium with and without sucrose at light intensities of 100 and 150 µmol $m^{-2}s^{-1}$. This is an important observation for photoautotrophic culture, in which an increased light intensity in the culture environment suppressed the effect of the need for sucrose on *M. elliptica* (Mart.) seedling regeneration. According to Kozai and Nguyen (2003), the light intensity must be increased to stimulate autotrophic behavior in seedlings *in vitro* when media devoid of sucrose are used. Light, as the primary energy source, is one of the most important environmental factors for growth, directly influencing the development of morphophysiological mechanisms for adaptation to light variation (Li and Kubota, 2009), such as through altering leaf structure (Zhang et al., 2003).

Although the *M. elliptica* (Mart.) seedlings regenerated in the absence of sucrose, increasing the light intensity did not affect the accumulated total and leaf dry weights (Figs 3c and 3d). Additionally, these characteristics presented lower values at all light intensities compared with the seedlings cultured in the medium with sucrose (Figs 3c and d). The seedlings cultured in the medium without sucrose reached a mean total dry weight of 13.6 mg, which was 2.5 times lower than the mean for the seedlings cultured in the presence of sucrose. These results can be explained by the fact that these plants only use their photosynthetic system as a way to accumulate carbon; however, alternative types of seals that result in greater gas exchange were not evaluated in the present study. According to Iarema et al. (2012), seals with membranes that allow greater ventilation within a flask must be used when performing cultures without sucrose supplementation. Greater ventilation within the *in vitro* culture flask allows a sufficient CO_2 concentration to ensure photosynthesis and seedling growth (Kitaya et al., 2005).
The plant leaf area is another characteristic related to the accumulated dry weight for this variable; a greater leaf area (2.408 cm^2) was observed when the *M. elliptica* (Mart.) seedlings were cultured in the presence of sucrose, while a smaller leaf area (1.67 cm^2) was observed in the absence of sucrose, regardless of the light intensity. This parameter is very important, as the leaf is responsible for the largest portion of carbohydrate production essential for plant growth and development (Marafon, 2012). This information corroborates the observed lower specific leaf area values associated with sucrose availability in the medium, representing a greater accumulated dry weight by area. A mean specific leaf area of 112.706 cm^2g^{-1} was obtained during culture in the presence of sucrose, while a value of 199.266 cm^2g^{-1} was recorded in the absence of sucrose.

Regarding the effect of light intensities on the specific leaf area, the seedlings showed the highest value (182.1 cm^2g^{-1}) at a light intensity of 0 µmol $m^{-2}s^{-1}$, and increasing light intensity decreased this parameter ($Y = -0.349x + 182.171$ $r^2 = 0.635$, $p \leq 5\%$). Low light levels can generally lead to an increased specific leaf area of the plant to intercept more radiation, a decreased leaf thickness and, thus, a decreased net assimilation rate (NAR), corresponding to an increased accumulated dry matter weight in the plant per available leaf
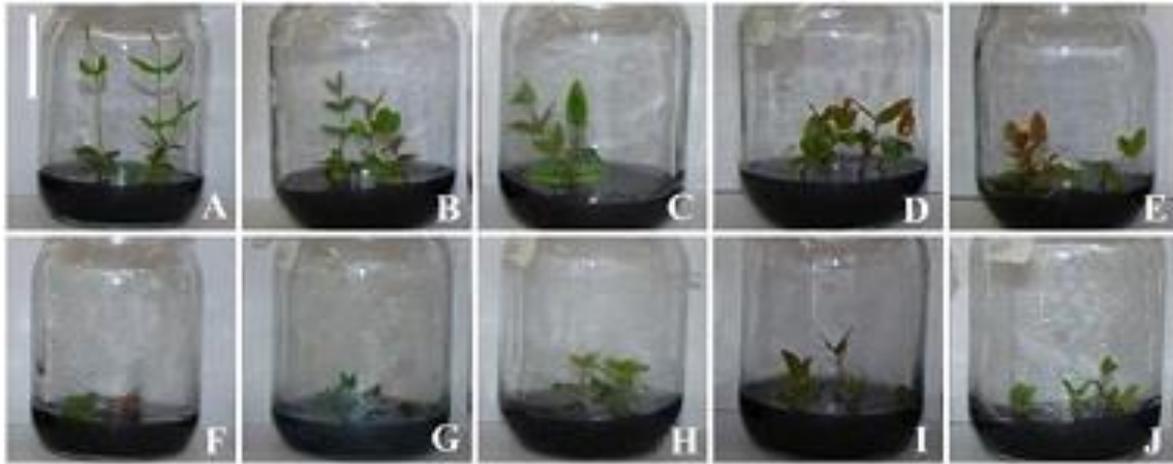
Fig 1. Growth of *M. elliptica* (Mart.) seedlings in culture medium supplemented with sucrose (A - E) and without sucrose (F - J) at light intensities of 0 µmol m^{-2}s^{-1} (panels A and F), 50 µmol m^{-2}s^{-1} (panels B and G), 75 µmol m^{-2}s^{-1} (panels C and H), 100 µmol m^{-2}s^{-1} (panels D and I), and 150 µmol m^{-2}s^{-1} (panels E and J) µmol m^{-2}s^{-1}. Scale bar = 2 cm.
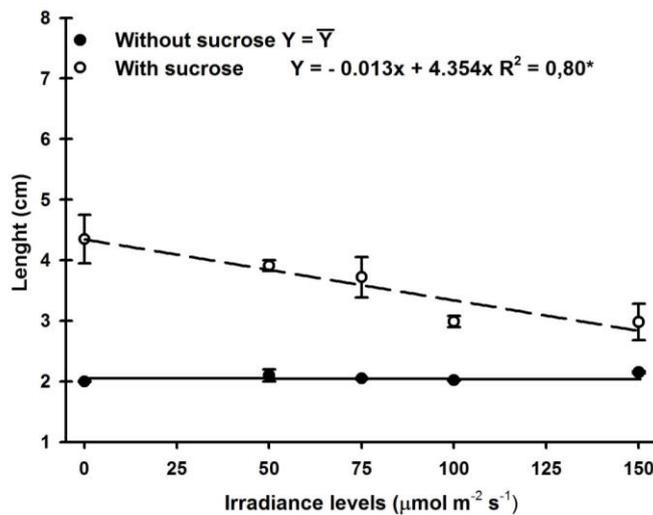
Fig 2. Lengths of *M. elliptica* (Mart.) seedlings in culture medium with and without sucrose at light intensities of 0, 50, 75, 100, and 150 µmol m^{-2}s^{-1}. *Significant at 0.05.

area unit (Marafon, 2012). Steinger et al. (2003) considered this an adaptation to meet photosynthetic demands. To test the maximum production of the shoots and leaves and the biomass accumulation in *M. elliptica* (Mart.) seedlings cultured in the absence of sucrose, the development of other studies involving a light supply above 150 µmol m^{-2}s^{-1} will be necessary. Additionally, studies that involve the development of cultures that allow greater gas exchange between the culture environment and the external atmosphere (Iarema et al., 2012) combined with high light intensities and alternative support for the culture medium (Saldanha et al., 2014) would be informative. These conditions characterize the photoautotrophic system (Xiao et al., 2011).

Anatomical characteristics: M. elliptica (Mart.) exhibits leaf plasticity

Studies that demonstrate the effect of different light intensities on the morphoanatomic characteristics of native Cerrado plants cultured *in vitro* are still scarce, especially when correlated with the presence or absence of sucrose in the culture medium. There are no available studies that demonstrate such characteristics for *M. elliptica* (Mart.). Morphoanatomic and physiological changes in the leaves are common plant adaptive responses to different environmental conditions (Pereira et al., 2013). The *M. elliptica* (Mart.) explants cultured in the absence of sucrose and light did not possess the ability to form tissues and did not regenerate new seedlings. Thus, the comparisons conducted in the micromorphometric analyses of the leaves of the seedlings cultured *in vitro* only correspond to light intensities of 50, 75, 100, and 150 µmol m^{-2}s^{-1}, together with the presence and absence of sucrose in the medium. There was a significant interaction between the light intensity and the culture medium for the chlorophyll parenchyma thickness (CP T), stomatal crypt density (St Cr Dn), stomatal crypt depth (St Cr Dp), and stomatal crypt opening area (St Cr O). An isolated effect of these characteristics was observed for the adaxial epidermis thickness (Ad Ep T) and abaxial epidermis thickness (Ab Ep T) (p ≤ 0.05). The presence of stomata in adaptive structures known as stomatal crypts (Figs 4a and 5 a-h) was noted, and the stomata were only identified on the
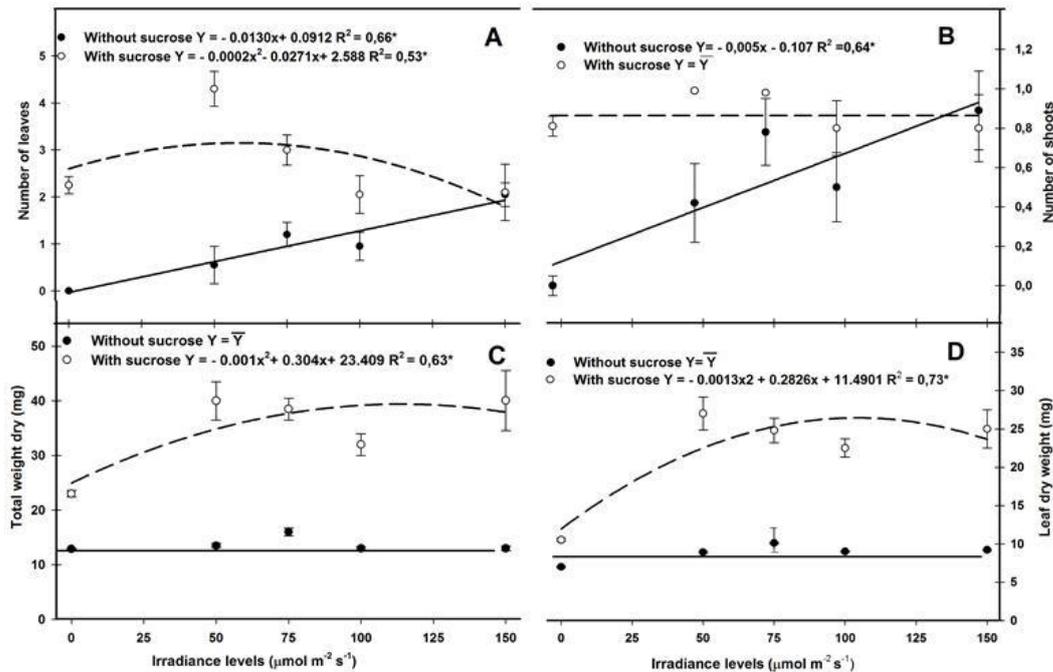
Fig 3. Number of leaves (A), number of shoots (B), total dry weight (C), and leaf dry weight (D) of *M. elliptica* (Mart.) seedlings cultured in media with and without sucrose at light intensities of 0, 50, 75, 100, and 150 µmol m^{-2}s^{-1}. *Significant at 0.05.
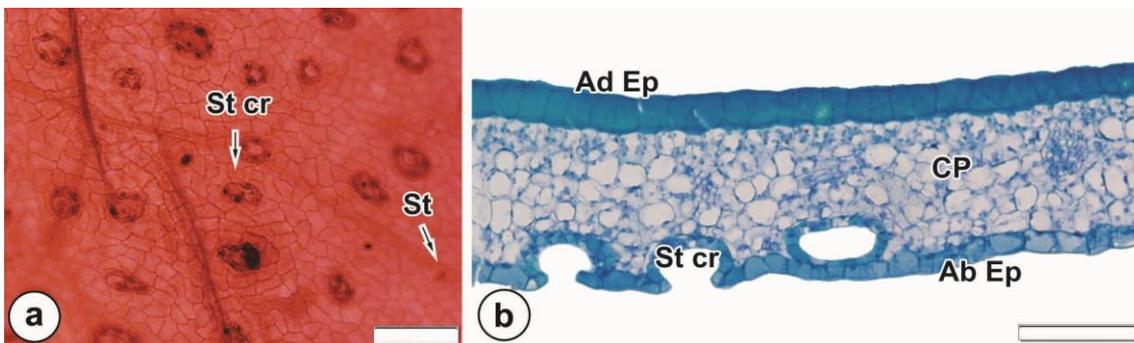
Fig 4. Photomicrographs of *M. elliptica* (Mart.) leaves *in vitro* in the absence of light and the presence of sucrose. (a) A portion of the abaxial epidermis with stomatal crypts (St Cr) and stoma (St) outside the stomatal crypt and (b) the cross-section of the blade's median region showing the cell arrangement in the adaxial epidermis (Ad Ep), chlorophyll parenchyma (CP), abaxial epidermis (Ab Ep), and stomatal crypt (St Cr). Scale bar = 100 µm.

abaxial surface; therefore, the plants can be classified as hypostomatic. The adaptive significance of the stomatal crypts is still under discussion, and they probably evolved in response to several environmental factors, most likely as a resource for xerophilic plants to reduce water loss via reduced leaf transpiration (Hassiotou et al., 2009). This concept is reinforced by the frequency of trichomes that are generally identified in the crypts (Rotondi et al., 2003; Jordan et al., 2008); however, trichomes were not observed in the plants under study. Stomata positioned in crypts may be more protected from environmental stressors than stomata located at the leaf surface (Haworth and McElwain, 2008). However, Roth-Nebelsick et al. (2009), who studied the functions of the stomatal crypts, concluded that future studies should focus on their effects on water vapor and CO_2 diffusion. Fig 5 a-h shows the distribution of the crypts on the abaxial surface of the *M. elliptica* (Mart.) leaves under different culture conditions. "Croada" leaves cultured in the presence of sucrose at a light intensity of 50 µmol m^{-2}s^{-1} exhibited a higher St Cr Dn, with a mean of 180 crypts/mm^2, and higher

light intensity resulted in a decrease in St Cr Dn (Fig 6d). At a light intensity of 150 µmol m^{-2}s^{-1}, there was no difference in the crypt density with or without sucrose, with means of 113.34 and 114.85 crypts/mm^2, respectively. Stomatal crypts were also identified in the leaves of the seedlings cultured in the dark with sucrose as the metabolic energy source (Fig 4a). However, the density of 75.56 crypts/mm^2 observed under these conditions was lower than that in the seedlings cultured in the light. A higher St Cr Dn may benefit the "croada" seedlings during the acclimatization process by providing greater control over gas exchange and enabling a reduction in water loss (Hassiotou et al., 2009). The chlorophyll parenchyma modified its structural organization according to the environment, ranging from homogenous, as observed in the leaves of seedlings cultured in the dark (Fig 4b), to dorsoventrally heterogeneous, with palisade parenchyma (columnar cells) located under the adaxial epidermis and spongy parenchyma (irregular shaped cells) under the abaxial epidermis (Fig 7a-h), demonstrating great leaf plasticity for adaptation to different environmental conditions. When the
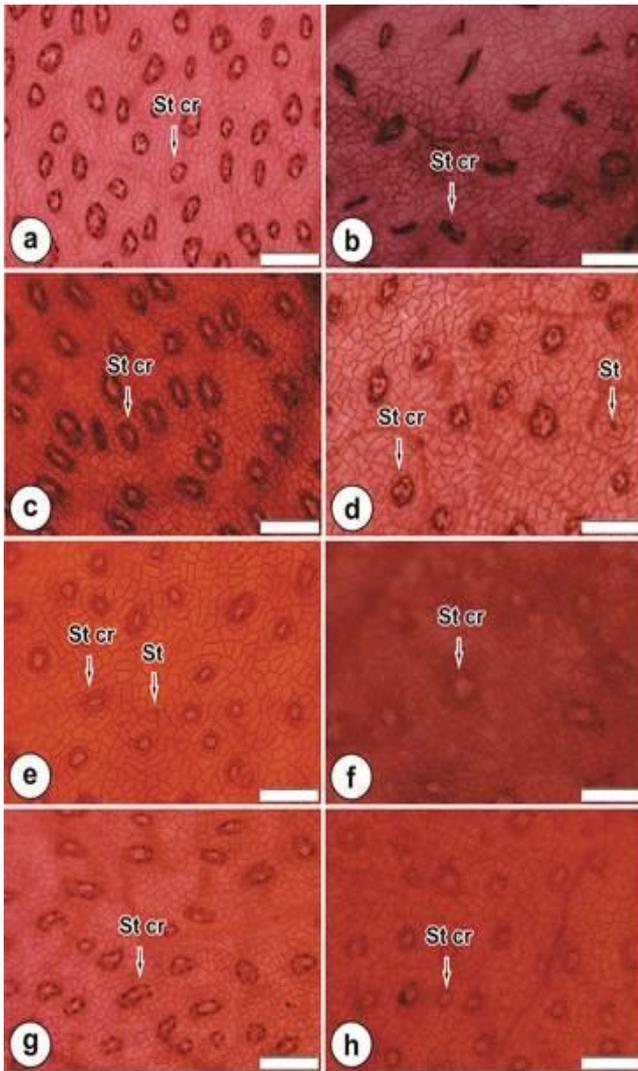
Fig 5. Photomicrographs of *M. elliptica* (Mart.) leaves *in vitro* showing the abaxial epidermis with stomatal crypts (St Cr) in the presence (1st column) and absence of sucrose (2nd column) and with increasing light intensity (**a**; **b** 50 μmol m^{-2}s^{-1}), (**c**; **d** 75 μmol m^{-2}s^{-1}), (**e**; **f** 100 μmol m^{-2}s^{-1}), and (**g**; **h** 150 μmol m^{-2}s^{-1}). Scale bar = 100 μm

palisade parenchyma is more developed, it facilitates the absorption of carbon dioxide (CO_2) into the mesophyll cells when they are directly exposed to light (Terashima et al., 2005). In addition, the palisade parenchyma can be responsible for reduced leaf heating, thus maintaining optimal temperatures for physiological processes (Taiz and Zeiger, 2009). The chlorophyll parenchyma thickness was greater in the absence of sucrose at all tested light intensities (Fig 6c). However, a greater total dry weight and leaf dry weight was observed in seedlings cultured in the presence of sucrose; these results can be explained by the accumulation of polysaccharides as starch grains within the cells (Figs 7a, 7c, 7e and 7g), which was not observed in the leaf tissues of the seedlings without sucrose (Fig 7b, 7d, 7f and 7h). Thus, supplying sucrose to the culture medium expanded the starch reserves of the micropropagated plants. In cross-sections of the *M. elliptica* (Mat.) leaves, a square-to-rectangular uniseriate adaxial and abaxial epidermis was observed (Fig 7a-h). The adaxial epidermis thickness (Ad Ep T) remained

unaffected by the different light intensities (Fig 6a); these results corroborate those obtained by Espindola-Júnior et al. (2009) in a study on *Mikania glomerata* Spreng. plants subjected to different light conditions. A difference in the Ad Ep thickness was only observed for the type of culture medium, with a value of 22.03 μm in the cultures devoid of sucrose and a mean thickness of 18.61 μm in the medium supplemented with sucrose. In a study by Santana et al. (2008) using a photoautotrophic stimulus culture system for *Annona glabra* L., a thicker epidermis formed on the adaxial surface compared with the epidermis that formed when a heterotrophic culture system was used. These authors identified characteristics of the plants that developed in the photoautotrophic environment similar to the characteristics of the plants grown *ex vitro*, which is considered an important factor in the acclimatization process. The abaxial epidermis thickness (Ab Ep T) varied according to the environmental energy supply (Fig 6c). A light intensity of 120 μmol m^{-2}s^{-1} induced a greater Ab Ep thickness in the "croada" leaves, regardless of the presence or absence of sucrose in the medium. Epidermis thickness is related to greater lignin synthesis in this tissue and is directly conditioned to environmental light, as light interferes with enzymatic activities, promoting the formation of phenylalanine and tyrosine. The enzymes in different tissues catalyze the deamination of these substances for the synthesis of aromatic monomer units, which are precursors of lignin (Abreu, 1994). At light intensities of 75 and 150 μmol m^{-2}s^{-1} in the absence of sucrose, the obtained St Cr O values were 560.38 and 340.25 μm, respectively, which were higher than the values observed in the presence of sucrose (244.276 and 175.095 μm, respectively). At light intensities of 50 and 100 μmol m^{-2}s^{-1}, there was no difference in the St Cr O values recorded in the absence (298.97 and 261.55 μm, respectively) and presence (296.31 and 213.92 μm, respectively) of sucrose. The stomatal crypt openings can be observed in Fig 5. Linear behavior ($Y = 29.364 + 0.1372x$, $r^2 = 0.908$) was observed for St Cr Dp as a function of the light levels in the absence of sucrose. Deeper stomatal crypts can facilitate CO_2 diffusion to assimilation sites (Roth-Nebelsick et al., 2009). None of the tested mathematical models fit the St Cr Dp data in the presence of sucrose.

Materials and Methods

Obtaining plant material and in vitro establishment

The nodal segments (2 cm long) with two axillary buds were removed from the *Mouriri elliptica* (Mat.) seedlings that were obtained from seeds and cultured in trays with sand. After obtaining the segments, they were disinfected under running water with three drops of neutral detergent for 15 minutes and 30 seconds in 70% alcohol and 15 minutes in a 0.5% commercial sodium hypochlorite. Following disinfection, the explants were inoculated in test tubes containing 20 mL of a culture medium with only water and agar; they were maintained in a growth room for 15 days at 25±2°C, under a photoperiod of 16/8 hours (light/dark), with light being provided by 40-Watt fluorescent lights. After this period, these explants were transferred to flasks containing 50 mL of wood plant medium (WPM) (Lloyd and Mccown , 1981) with 50% salt and 2 g of activated charcoal and solidified with 3.5 gL^{-1} of agar. The pH of the culture medium was adjusted to 5.7±0.03 prior to autoclaving at 121°C for 20 minutes. PVC film was used to seal the flasks after inoculation.
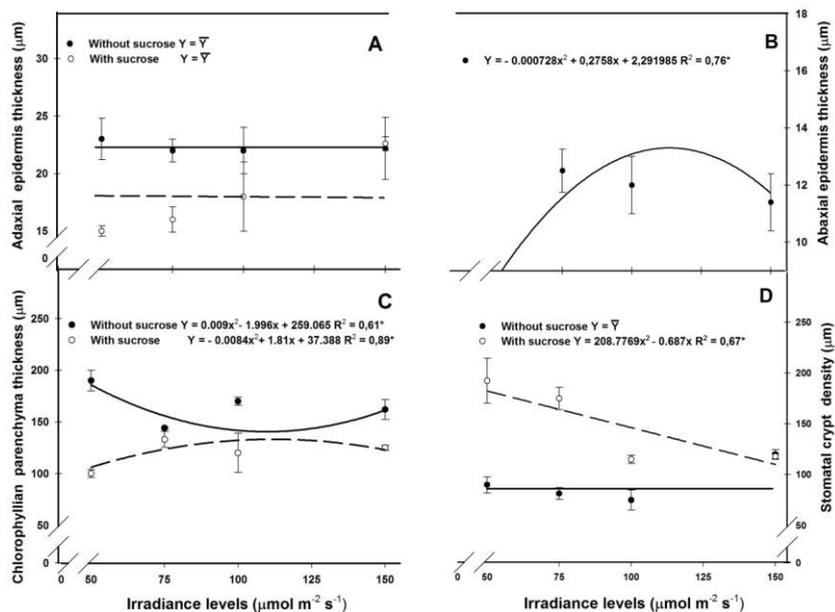
Fig 6. Adaxial epidermis thickness (A), abaxial epidermis thickness (B), chlorophyllin parenchyma thickness (C), and stomatal crypt density (D) of M. elliptica (Mart.) seedlings cultured in media with and without sucrose at light intensities of 0, 50, 75, 100, and 150 µmol m^{-2}s^{-1}. *Significant at 0.05.
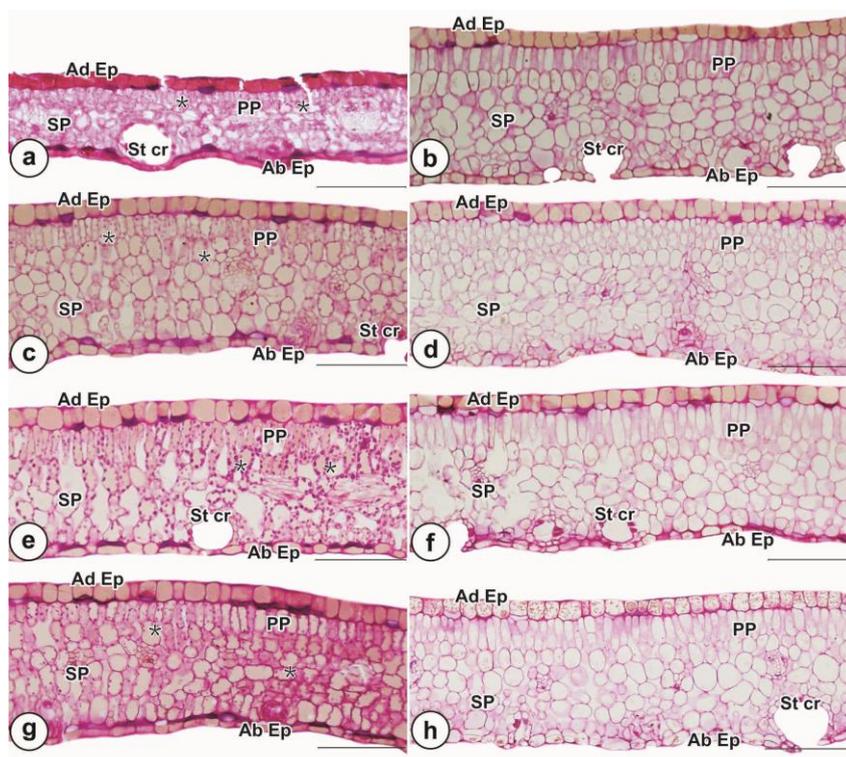
Fig 7. Photomicrographs of cross-sections of the median region of M. elliptica (Mart.) leaves in vitro showing the cellular arrangement of the adaxial epidermis (Ad Ep), palisade parenchyma (PP), spongy parenchyma (SP), abaxial epidermis (Ab Ep), and stomatal crypts (St Cr) in the presence (1st column) and absence of sucrose (2nd column) at increasing light intensity (a; b 50 µmol m^{-2}s^{-1}), (c; d 75 µmol m^{-2}s^{-1}), (e; f 100 µmol m^{-2}s^{-1}), and (g; h 150 µmol m^{-2}s^{-1}). *Polysaccharides accumulated within the cells; tissues were stained via the PAS method. Scale bar = 100 µm.

In vitro culture of nodal segments of *M. elliptica* (Mart.)

Two types of medium were used: with and without 30 gL^{-1} of sucrose. To test the effect of light intensities of 0, 50, 75, 100, and 150 $\mu mol\ m^2s^{-1}$ on the *in vitro* culture of *Mouriri elliptica* (Mat.), the flasks were placed in a climatic chamber (Fitotron®) at 25°±2°C with 60% relative humidity. The light levels were adjusted using a QSO-S photosynthetically active radiation sensor (Decagon Devices, Pullman, WA, USA).

Growth evaluation

Evaluations were performed after 60 days of *in vitro* culture. The following parameters were evaluated: the seedling length (cm), the number of shoots and leaves, the total and leaf dry weights (mg), the leaf area (cm^2), and the specific leaf area (cm^2g^{-1}). The leaf area was determined through image integration using image analysis software (ImageJ®). The length measurements were obtained using a millimeter ruler. The total dry weight and leaf dry weight were determined using a digital analytical balance after drying the material in a forced air oven at 65ºC for 72 hours. The specific leaf area was obtained from the ratio between the leaf area (cm^2) and the leaf dry weight (grams).

Anatomical characterization

For the anatomical analyses, the leaf samples were fixed in Karnovsky solution (Karnovsky, 1965) for 48 hours and then dehydrated in an ascending ethanol series, pre-infiltrated, and infiltrated with historesin (Historesin Leica, Erviegas Ltda: São Paulo - SP, Brazil) according to the manufacturer's recommendations. After the blocks were dried, the material was transversely sectioned into 5-µm-thick samples in a rotary microtome (RM 2155 model, Leica). The sectioned material was stained with 0.05% toluidine blue, pH 4.0 (O'Brien et al., 1965), to evaluate the epidermis thickness of both surfaces, the chlorophyll parenchyma thickness and the depth of the stomatal crypts. (St Cr Dp). The periodic acid-Schiff (PAS) reaction was used to observe neutral polysaccharides. The PAS reaction was controlled through the acetylation of the material or via the omission of oxidation by periodic acid (McManus, 1948). The diaphanization technique was used to determine the density of the stomatal crypts (St Cr Dn) of the leaf surface and the crypt opening area. For this purpose, leaf samples were immersed in 5% sodium hydroxide for 24 hours and then clarified with chloral hydrate (1.6:1, p/v) for 24 additional hours and stained with 1% safranin in 50% ethanol (Arnott, 1959). Images were obtained under an optical microscope (BX61 model, Olympus) using the U-photo system in theLaboratory of Plant Anatomy (Laboratório de Anatomia Vegetal) of the Goiás Federal Institute of Education, Science, and Technology – Rio Verde Campus, Brazil.

Statistical analysis

The experiment was arranged in a completely randomized design (CRD) under a 2x5 factorial scheme with two types of culture medium (with and without 30 gL^{-1} of sucrose) and five different light intensities (0, 50, 75, 100, and 150 $\mu mol\ m^2s^{-1}$), with four replicates and four explants per flask. The data were subjected to analysis of variance (ANOVA) using the F test in addition to the comparison of means using Tukey's test (5% probability) for qualitative factors and regression analysis at the 5% probability level for quantitative factors.

Conclusion

It was possible to regenerate *Mouriri elliptica* (Mart.) seedlings in the absence of sucrose by providing a higher light intensity (at least 50 $\mu mol\ m^{-2}s^1$) to the culture environment. However, better seedling performance was obtained when sucrose was used as the metabolic energy source. The species under study exhibits great leaf plasticity when cultured under photoautotrophic conditions. Thus, the plants show a great ability to adapt to environmental variation, especially regarding light.

Acknowledgments

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