



# Dissimilarity between *Mouriri elliptica* (Mart.) plants cultivated *in vitro* and *in situ* through anatomic parameters

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**ABSTRACT.** *Mouriri elliptica* (Mart.) is a genetic resource of the Cerrado domain, which has potential for food and medicinal use. A few studies have been performed on its *in vitro* propagation, and no studies have examined dissimilarities between plants of this species when cultivated *in situ* or *in vitro*. Therefore, the objective of the present study was to identify *in vitro* cultivation conditions that permit the formation of plantlets with leaf anatomical features that are less dissimilar to those of plants grown *in situ*. Thus, an anatomical study of the leaves was conducted to investigate the adaxial epidermis thickness, abaxial epidermis thickness, chlorenchyma thickness, stomatal crypt depth, stomatal crypt density, and leaf surface stomatal crypt aperture area. The distance between phenotypes was determined based on micromorphometric data, and unweighted pair group mean arithmetic clustering was performed. Four different groups were tested,

and cultivation with sucrose and 50 and 75  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  irradiance was found to promote plantlet development and maximize similarity to plants cultivated *in situ*. The most important anatomical parameters in this study were the stomatal crypt aperture area and the crypt density. This study is of importance for the anatomical characterization of *M. elliptica* (Mart.) leaves, as it identifies plasticity as a function of *in vitro* culture conditions.

**Key words:** Leaf micromorphometrics; Micropropagation; Phenotype; Plantlets; UPGMA clustering

## INTRODUCTION

*Mouriri elliptica* (Mart.) (Melastomataceae family) is a native plant of the Cerrado domain and is a potential resource for human populations. It produces nutritious fruits that contain antioxidant compounds, which can be consumed *in natura* or processed into jam (Rufino et al., 2010; Rufino et al., 2011). *M. elliptica* leaves and/or bark have been used to treat gastric ulcers and gastritis, and they may also have an antimicrobial effect. These medicinal effects stem from stimulation of the immune system in response to pathogens (Moleiro et al., 2009; Vasconcelos et al., 2010b) and from the inhibition of the oxidative capacity of *Helicobacter pylori* (Bonacorsi et al., 2013).

The sexual reproduction of *M. elliptica* (Mart.) is very complex, due in part to the presence of a hard tegument in its seeds that renders germination difficult (Vasconcelos et al., 2010a; Lima et al., 2016). Thus, it is necessary to utilize practices that overcome dormancy or that obtain seedlings through vegetative propagation. Plant tissue culture has been an indispensable tool for obtaining seedlings of various native and cultivated species, such as *Anacardium othonianum* Rizz (Assis et al., 2015), *Byrsonima cydoniifolia* A. Juss. (Martendal et al., 2013; 2014), *Pfaffia glomerata* (Spreng.) Pedersen (Saldanha et al., 2014), and banana (*Musa* spp AAA) plantlets (Kaçar et al., 2010).

During *in vitro* cultivation, it is important to obtain plantlets that have characteristics similar to those of plants grown *in situ*. *In vitro* cultivation conditions normally permit rapid plant growth and multiplication, but may also induce structural and physiological changes that render plants unfit to survive adverse environmental conditions (Rout et al., 2006). Thus, photoautotrophic micropropagation has been investigated, in which the absence of sucrose in the growth medium and increased irradiance from the environment are used to promote the formation of plantlets with characteristics that benefit their survival during acclimatization (Xiao and Kozai, 2006; Iarema et al., 2012; Sáez et al., 2012; Assis et al., 2016).

To determine whether plantlets that are micropropagated in a photoautotrophic system develop morphophysiological characteristics similar to those of *in situ* plants, the use of multivariate techniques is appropriate. According to Cruz et al. (2011), multivariate analysis has diverse applications, meeting the needs of investigators with a wide range of interests and knowledge. This technique is commonly used in plant breeding programs (Silva et al., 2015) as a way to evaluate genetic diversity (de Assis et al., 2013) or superior genotypes (Oda et al., 2015), and to conduct environmental studies (Di Leo et al., 2015; Ma et al., 2016).

To our knowledge, no studies to date have used multivariate analysis for the comparative evaluation of *M. elliptica* (Mart.) plants cultivated *in vitro* and *in situ* based

on anatomical features. Anatomical features may support future studies to characterize the genetic diversity among individuals and populations of this species. Thus, this study aimed to use multivariate statistical techniques to evaluate the dissimilarity between plants cultivated *in vitro* and *in situ*.

## MATERIAL AND METHODS

### Plant material and *in vitro* cultivation conditions

The study material consisted of leaves from mature plants of *M. elliptica* (Mart.) grown under natural conditions (*in situ*) and leaves of plantlets cultivated *in vitro*. Both the leaves of mature plants and the seeds from which to grow plantlets were collected on a private property located in the Planalto Verde District, municipality of Montividiu, Goiás, Brazil (17°19.201'S, 51°33.500'W and 982 m in altitude).

Before implementing the experiment, 2-cm long nodal segments with two axillary buds each were inoculated in test tubes containing 20 mL growth medium composed of water and agar. The tubes were kept in a growth room for 15 days at  $25^{\circ} \pm 2^{\circ}\text{C}$  with a photoperiod of 16/8 h (light/dark), under irradiance from 40-W fluorescent lamps. After that period, the explants were transferred to vials containing 50 mL wood plant medium (WPM) (Lloyd and McCown, 1981) containing 50% salts and 2.5 g/L added activated carbon solidified with 3.5 g/L agar, with or without 30 g/L sucrose. The pH of the growth medium was adjusted to  $5.7 \pm 0.03$  before autoclaving at  $121^{\circ}\text{C}$  for 20 min. The vials were sealed after inoculation with PVC plastic film.

The experiment was organized with a completely randomized design in a growth chamber (Fitotron®) at  $25^{\circ} \pm 2^{\circ}\text{C}$  and with a relative humidity of 60%. Irradiance of 0, 50, 75, 100, and  $150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  was evaluated and adjusted based on periodic tests with the aid of a QSO-S photosynthetically active radiation sensor (Decagon Devices, Pullman, WA, USA).

As no plantlets were formed in the absence of both sucrose and light, nine *in vitro* cultivation conditions were used in the study, as follows: C1, sucrose and zero irradiance; C2, sucrose and  $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; C3, sucrose and  $75 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; C4, sucrose and  $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; C5, sucrose and  $150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; C6, absence of sucrose and  $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; C7, absence of sucrose and  $75 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; C8, absence of sucrose and  $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; and C9, absence of sucrose and  $150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . These conditions were compared and correlated with each other and with those of *in situ* plants (CO).

### Anatomical study of *M. elliptica* (Mart.) leaves

The leaves of *M. elliptica* (Mart.) were subjected to fixation and diaphonization. For fixation, leaves were submerged in Karnovsky's solution (Karnovsky, 1965) for 48 h, dehydrated in a graded ethanol series, and pre-infiltrated and infiltrated with Histo-resin (Leica) according to the manufacturer instructions. The material was used to generate 5-mm sections with a rotary microtome (RM 2155, Leica). The sections were stained with 0.05% toluidine blue at pH 4.0 (O'Brien et al., 1965).

For diaphonization, leaf samples were immersed in a 5% sodium hydroxide solution for 24 h, clarified with chloral hydrate (1.6:1, p/v) for a further 24 h, and stained with 1% safranin in 50% ethanol (Arnott, 1959). Slides containing the material were cover slipped using Canada balsam.

Images were obtained under an optical microscope (BX61, Olympus) with a U-photo system at the Plant Anatomy Laboratory of Instituto Federal de Educação, Ciência e Tecnologia Goiano, Rio Verde Campus. The adaxial epidermis thickness (Ad Ep T), abaxial epidermis thickness (Ab Ep T), chlorenchyma thickness (Ch T), stomatal crypt depth (St Cr Dp), stomatal crypt density (St Cr D), and leaf-surface stomatal crypt aperture area (St Cr A) were evaluated. Four fully formed leaves were extracted from three randomly chosen plants grown *in situ* or for each cultivation in the *in vitro* condition. Ten images per repetition were evaluated (leaf tissue) for each feature, totaling 40 measurements per plant.

### Statistical analysis

To determine dissimilarity between plants grown *in situ* and *in vitro*, the data were subjected to analysis of variance (ANOVA) by F testing at a 5% probability. Based on the results of ANOVA, a variance matrix and residual covariance were obtained. The dissimilarity matrix between the plant growth conditions was then determined by the generalized Mahalanobis distance, while the relative contribution of the micromorphometric features (S<sub>j</sub>) was obtained according to the method described by Singh (1981), using the software GENES (Cruz, 2013). Subsequent clustering was conducted by the average linkage between groups (unweighted pair groups mean arithmetic, UPGMA) using the cluster package in R (Maechler, 2010). To determine clustering accuracy, the cophenetic correlation coefficient (CCC) was calculated, which was obtained with 1000 simulations with the help of the GENES software (Cruz, 2013). A descriptive analysis of the anatomical features was also conducted.

## RESULTS

### Analysis of dissimilarity between *M. elliptica* (Mart.) plants grown *in situ* and *in vitro*

ANOVA results are presented in Table 1. Differences were observed between *M. elliptica* (Mart.) plants cultivated *in vitro* and *in situ* for all traits investigated in this study. The coefficient of variation was between 7.39 and 24.26%, demonstrating good experimental consistency.

**Table 1.** Summary of the analysis of variance informing the mean square, mean, and coefficient of variation (CV) of the anatomical features of *Mouriri elliptica* (Mart.).

FV	d.f.	Mean square					
		Ad Ep	Ch T	Ab Ep T	St Cr Dp	St Cr A	St Cr Dp
Tr	9	23.39*	5862.03**	10.55**	185.59**	37055.13**	6041.56**
Re	20	7.86	206.84	1.83	9.42	4738.05	407.31
Mean		19.65	141.82	11.22	41.49	283.63	118.79
CV (%)		14.27	10.14	12.05	7.39	24.26	16.98

Ad Ep T, adaxial epidermis thickness (µm); Ch T, chlorenchyma thickness (µm); Ab Ep T, abaxial epidermis thickness (µm); St Cr Dp, stomatal crypt depth (µm); St Cr A, stomatal crypt aperture area (µm); St Cr D, stomatal crypt density (crypts/mm<sup>2</sup>), were evaluated in leaves of *M. elliptica* (Mart.). \*\*\*Significance at 0.01 and 0.05, respectively, as determined by the F test.

Estimates of phenotypic correlations among the six micromorphometric features of leaves are shown in Table 2. A strong but negative correlation (-0.73) was identified between the characteristics Ad Ep T and St Cr D; therefore, cultivation conditions that provide higher St Cr D tend to form plantlets with thinner Ad Ep T. A positive correlation (0.52) was observed

between the parameters St Cr Dp and St Cr D. Based on the positive correlation of 0.47, plantlets with thicker chlorenchyma tended to develop deeper stomatal crypts, representing acclimatization of the plantlet to the cultivation condition.

**Table 2.** Phenotypic correlation coefficients ( $r_p$ ) between micromorphometric features leaves.

Features	Ad Ep T	Ch T	Ab Ep T	St Cr Dp	St Cr A	St Cr D
Ad Ep T	1					
Ch T	0.267	1				
Ab Ep T	0.052	-0.340	1			
St Cr Dp	0.072	0.478	0.354	1		
St Cr A	0.330	-0.046	-0.233	-0.053	1	
St Cr D	-0.737	0.209	0.102	0.521	-0.415	1

Ad Ep T, adaxial epidermis thickness; Ab Ep T, abaxial epidermis thickness; Ch T, chlorenchyma thickness; St Cr D, stomatal crypt density; St Cr Dp, stomatal crypt depth; St Cr A, stomatal crypt aperture area.

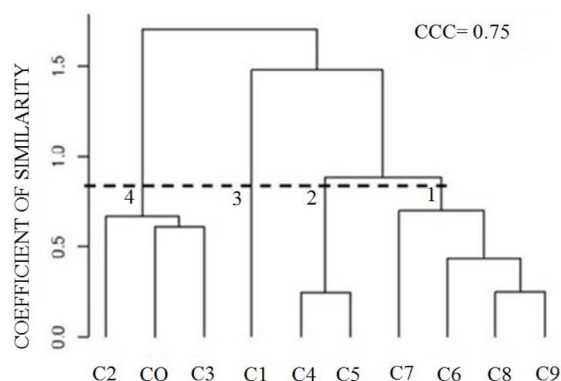
Dissimilarities between *M. elliptica* (Mart.) plants in each of the 10 cultivation conditions ranged from 0.2 to 2.7 (Table 3). Plantlets micropropagated under C2 (sucrose and irradiance of 50  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) and C3 (sucrose and irradiance of 75  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) conditions were less dissimilar to *in situ* plants, with observed dissimilarities were 0.7 and 0.6, respectively. A higher dissimilarity (2.7) was observed between *in situ* plants and C1 (sucrose and absence of light) or C7 (absence of sucrose and irradiance of 75  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) conditions. A lower dissimilarity (0.2) was observed between the *in vitro* cultivation conditions C4 (sucrose and irradiance of 100  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) and C5 (presence of sucrose and irradiance of 150  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ).

**Table 3.** Dissimilarity matrix obtained by the generalized Mahalanobis distance ( $D^2$ ) between *Mouriri elliptica* (Mart.) plantlets grown under different *in vitro* and *in situ* cultivation conditions.

Environment	CO	C1	C2	C3	C4	C5	C6	C7	C8	C9
CO	0									
C1	2.7	0								
C2	0.7	1.3	0							
C3	0.6	2.1	0.6	0						
C4	1.3	0.8	1.1	0.7	0					
C5	1.8	1.7	1.9	0.9	0.2	0				
C6	1.9	1.5	1.9	2.4	0.9	1.6	0			
C7	2.7	1.7	2.2	2.1	1.03	1.3	0.9	0		
C8	1.7	1.2	1.7	1.6	0.4	0.6	0.3	0.6	0	
C9	1.2	1.8	1.3	1.1	0.5	0.7	0.6	0.5	0.3	0

CO, *in situ*; C1, sucrose and zero irradiance; C2, sucrose and 50  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; C3, sucrose and 75  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; C4, sucrose and 100  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; C5, sucrose and 150  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; C6, absence of sucrose and 50  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; C7, absence of sucrose and 75  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; C8, absence of sucrose and 100  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; C9, absence of sucrose and 150  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  irradiance.

Based on the dissimilarity matrix among the 10 *M. elliptica* (Mart.) cultivation conditions, it was possible to identify UPGMA clustering. The CCC was 0.75, demonstrating consistency between the original dissimilarity values and those represented by the dendrogram. The cultivation conditions caused anatomical changes in the leaf that were responsible for the discrepancies observed among test plants. Thus, four distinct groups of plants were identified, with a dendrogram cut of approximately 50% (Figure 1).



**Figure 1.** Unweighted pair groups mean arithmetic (UPGMA) clustering of 10 *Mouriri elliptica* (Mart.) phenotypes. Dashed line: dendrogram cut indicating approximately 50% dissimilarity. CCC, cophenetic correlation coefficient; CO, *in situ* plantlets; C1 to C9, plantlets grown *in vitro*, under the following conditions: C1, sucrose and zero irradiance; C2, sucrose and 50  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; C3, sucrose and 75  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; C4, sucrose and 100  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; C5, sucrose and 150  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; C6, absence of sucrose and 50  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; C7, absence of sucrose and 75  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; C8, absence of sucrose and 100  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; C9, absence of sucrose and 150  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  of irradiance.

Plantlets micropropagated under photoautotrophic growth conditions grouped into the same cluster (1), which is consistent with the plantlet responses, as they were grown in the absence of sucrose (Figure 1). Phenotypic characteristics similar to those of *in situ* plants were observed in plantlets grown under the photomixotrophic conditions, C2 (sucrose and irradiance of 50  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) and C3 (sucrose and irradiance of 75  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) (Figure 1), with the latter being the most similar to *in situ* plants.

Plantlets grown in the presence of sucrose but without light (C1) had greater differences to those grown under other conditions in terms of their anatomical features, and they therefore formed their own group (3). Irradiance at 100 and 150  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  resulted in the development of plantlets with very similar anatomical features, both in the presence (C4 and C5) and absence of sucrose (C8 and C9) (Figure 1).

The relative importance of each assessed anatomical feature is shown in Table 4. The St Cr A was identified as the most important feature in the cluster study of *M. elliptica* (Mart.) plants *in situ* and under different *in vitro* cultivation conditions, with a contribution of 75.34%. The St Cr D and Ch T also contributed 12.28 and 11.92% of the clustering, respectively. The features Ad Ep T, Ab Ep T, and St Cr Dp were less important, with clustering contributions of 0.05, 0.02, and 0.37%, respectively.

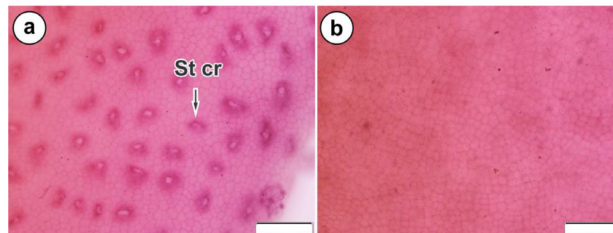
**Table 4.** Relative importance ( $S_j$ ) of micromorphometric features in the study of divergence study of in *Mouriri elliptica* (Mart.) plants grown *in situ* and plantlets subjected to different *in vitro* cultivation conditions.

Parameters	$S_j$	$S_j$ (%)
St Cr A ( $\mu\text{m}$ )	1,111,653.92	75.35
St Cr D ( $\text{mm}^2$ )	181,247.03	12.28
Ch T ( $\mu\text{m}$ )	175,860.93	11.92
St Cr Dp ( $\mu\text{m}$ )	5,567.96	0.37
Ad Ep T ( $\mu\text{m}$ )	701.93	0.05
Ab Ep T ( $\mu\text{m}$ )	316.70	0.02

St Cr A, crypt aperture area; St Cr Dp, stomatal crypt depth; Ch T, chlorenchyma thickness; St Cr D, stomatal crypt density; Ad Ep T, adaxial epidermis thickness; and Ab Ep T, abaxial epidermis thickness.

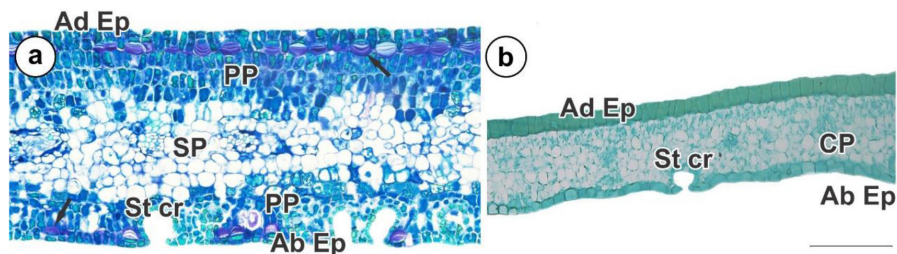
### Anatomic descriptions of *M. elliptica* (Mart.) leaves *in situ* and *in vitro*

The leaves of *M. elliptica* (Mart.), both *in situ* and *in vitro*, contain stomata within stomatal chambers called stomatal crypts (Figure 2a). Stomatal crypts were observed only on the abaxial surface of the leaves, classifying them as hypostomatic. The adaxial epidermal cells of the leaves developed an overlapping tetrahedral shape, regardless of the plant growth condition (Figure 2b).



**Figure 2.** Photomicrographs of a *Mouriri elliptica* (Mart.) leaf from a plant grown *in situ*. Abaxial epidermis with stomatal crypts (St Cr) (a) and adaxial epidermis (b). Scale bar = 100 µm.

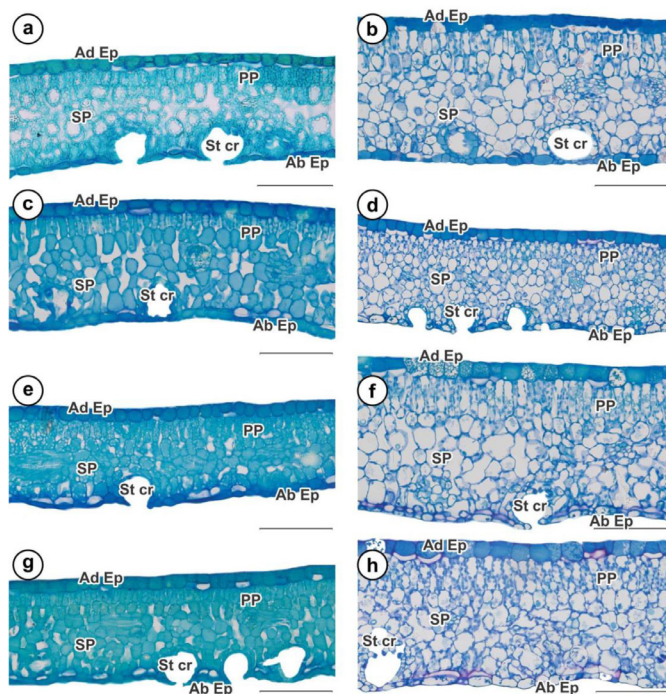
Plasticity was observed in the development of the chlorenchyma. Plants grown *in situ* (CO) developed isobilateral chlorenchyma, with layers of palisade cells facing both the adaxial and abaxial surfaces, and with spongy parenchyma between the two regions of palisade cells (Figure 3a). In plantlets grown in the presence of sucrose but without light (C1), chlorenchyma stratification was not observed; instead, the chlorenchyma was homogeneous (Figure 3b). The leaves of plants grown *in situ* possessed epidermal cells on the adaxial and abaxial surfaces that contained mucilage, which was colored purple by toluidine blue staining of the leaf tissue; however, this feature was not observed in the leaves of plantlets grown under the C1 condition (Figure 3a and b).



**Figure 3.** Cross sections of the middle region of *Mouriri elliptica* (Mart.) leaves grown *in situ* (a) and *in vitro* in the presence of sucrose and the absence of light (b). Toluidine blue was used to stain the leaf tissue. Ad Ep, adaxial epidermis; Ab Ep, abaxial epidermis; PP, palisade parenchyma; SP, spongy parenchyma; St Cr, stomatal crypt; and CP, chlorenchyma. The arrows indicate cells containing mucilage. Scale bars = 100 µm.

In plantlets grown under *in vitro* conditions (in the presence or absence of sucrose in the growth medium) with irradiance starting at  $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , dorsiventral chlorenchyma was observed, with 2-3 layers of palisade parenchymal cells facing the adaxial surface (Figure 4a-h). Spongy parenchyma, with more space between cells, was observed in the leaves of plants grown in the presence of sucrose, irrespective of the light intensity (Figure 4a, c, e, and g). Epidermal cells containing mucilage were also observed in plants from the *in vitro* cultivation

conditions with irradiance above  $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; however, there was only light toluidine blue staining in these cells, possibly due to reduced mucilage accumulation (Figure 4a-h).



**Figure 4.** Cross sections of the middle region of *Mouriri elliptica* (Mart.) leaves in the presence (left column) or absence (right column) of sucrose; rows show ascending irradiance levels (a. and b.,  $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; c. and d.,  $75 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; e. and f.,  $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; and g. and h.,  $150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ). Ad Ep, adaxial epidermis; Ab Ep, abaxial epidermis; PP, palisade parenchyma; SP, spongy parenchyma; and St Cr, stomatal crypt. Scale bars,  $100 \mu\text{m}$ .

## DISCUSSION

Anatomical plasticity between *M. elliptica* (Mart.) plantlets grown *in vitro* and plants grown *in situ* generated four distinct groups after UPGMA clustering. It was possible to estimate the dissimilarity between *M. elliptica* (Mart.) leaves grown *in situ* and *in vitro* based on phenotypic variations in the micromorphometric data. According to Cruz et al. (2011), phenotypic characteristics typically follow a continuous distribution and are determined by many genes that have small individual contributions, and are also influenced by the environment. Thus, it was possible to determine which *in vitro* cultivation conditions resulted in leaf development with anatomical characteristics that were less dissimilar to those of *in situ* plants.

The leaf anatomic characteristics observed in the *in vitro* plantlets are important for adaptation to growth conditions as they influence physiological processes, especially the ability to perform photosynthesis. In plantlets grown in the presence of sucrose but without light (C1), there was no stratification of the chlorenchyma, which instead, appeared homogeneous, thus demonstrating little tissue differentiation. Light intensities greater than  $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (C2 to C9) facilitated better leaf development, with stratification of the chlorenchyma into palisade



and spongy zones of the dorsiventral type. Anatomical plasticity in leaves of *M. elliptica* (Mart.) in response to cultivation conditions represents the acclimatization capacity of the species.

Stomatal crypts are an important feature of *in situ* plants, which were also observed in *in vitro* plants. Stomatal crypts are considered to be features that characterize species in their natural habitats, and many species are found in arid environments (Jordan et al., 2008). Crypts favor the development of plants in such environments as they restrict transpiration, which reduces water loss and promotes gas exchange at appropriate times (Hassiotou et al., 2009).

The anatomical parameters St Cr A and St Cr D together accounted for 87.63% of the relative importance (S<sub>j</sub>) in the UPGMA cluster. Therefore, those characteristics were considered to be key factors in the dissimilarity between *M. elliptica* (Mart.) plants cultivated *in situ* and *in vitro*. Four different groups were obtained with UPGMA clustering based on a dendrogram cut indicating approximately 50% dissimilarity. The CCC was 0.75, indicating that the clustering was consistent. Silva and Dias (2013) consider the assessment of cluster consistency by the CCC very important so that the conclusions on similarities between individuals may be considered trustworthy. According to Cruz and Carneiro (2006), a higher CCC value corresponds to lower clustering distortion.

None of the *in vitro* conditions used in this study permitted the formation of plantlets with anatomical features that were similar to those of *in situ* plants. However, plantlets cultivated under the C2 and C3 conditions, both of which were photomixotrophic, were less dissimilar to *in situ* plants. This suggests that these plants have the highest chances of survival when subjected to *ex vitro* conditions.

The first study on micropropagation of the species *M. elliptica* (Mart.) revealed the regeneration ability of plantlets under photoautotrophic conditions subjected to irradiance above 50  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Assis et al., 2016). In the present study, clustering analysis between *in situ* and *in vitro* plantlets based on leaf anatomical features showed that plantlets cultivated under a photoautotrophic system were more dissimilar to *in situ* plantlets. However, more studies on micropropagation of this species should be conducted in which plants are cultivated until they reach the acclimatization stage to ensure their survivability. This was observed by Corrêa et al. (2015) on the interactions between *P. glomerata* genotypes (Spreng.) in photoautotrophic culture, and by studies by Rodrigues et al. (2015) with *Etilingera elatior* (Jack) R.M. Smith (torch ginger).

Several studies on plant micropropagation have been performed to obtain plantlets with anatomical and physiological characteristics that increase their ability to survive the acclimatization process, which is a stressful stage for the plant. Among the developed studies, it is cited as an example with native plants *B. cydoniifolia* A. Juss. (Martendal et al., 2014), *Billbergia zebrina* (Martins et al., 2015), and cultivated plants of commercial importance as *Carica papaya* L. var. Red and Maradol (Pérez et al., 2015).

In conclusion, the anatomical characteristics studied in the leaves of *M. elliptica* (Mart.) support dissimilarity between plants grown *in situ* and those cultivated *in vitro* under photomixotrophic and photoautotrophic conditions. UPGMA clustering showed that *in vitro* cultivation conditions in the presence of sucrose with irradiance of 50 and 75  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  supported the growth of plantlets with leaf anatomical features that were less dissimilar to those of *in situ* plants placed in the same group.

## Conflicts of interest

The authors declare no conflict of interest.

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