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Morphoanatomical and biochemical responses of *Myracrodruon urundeuva* under photomyxotrophic culture, a native species with priority for local conservation

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Abstract
Photoautotrophy is one of the main tendencies *in vitro* cultivation, being considered economically efficient since it reduces the chemical inputs used in the culture medium and significantly improves the quality of the seedlings that will be taken to the field. Reforestation and environmental restoration programs involving tree species can benefit from the proper use of photoautotrophic micropropagation. *Myracrodruon urundeuva* Allemão is a tree with great socioeconomic value due to its chemical properties, having multiple uses by local communities, which raises the need for studies to support the production of seedlings of this species. In this sense, the present research aimed to evaluate the effect of different sucrose concentrations and seal types on the growth and morphophysiological quality of *M. urundeuva in vitro*, to reduce production costs and improve the quality of micropropagated seedlings. *M. urundeuva* diaspores were inoculated into test tubes containing 10 ml of Wood Plant Medium at 50% of the salt concentration. Three sucrose concentrations (0.0, 7.5 and 15.0 $g.L^{-1}$) combined with three seal types: total with cap, total with 3M® microporous membrane and partial seal were tested. The fresh weight, height, and leaf area were evaluated and the content of the photosynthetic pigment was analyzed, as well as morphoanatomical parameters. The CO_2 concentration was higher in the microporous membrane sealed tubes. The total microporous membrane system favored increase in the growth, and when took out the sucrose the increased the carotenoids contents. The density of tector trichomes was influenced only by the sealing factor. Seedlings cultivated without sucrose in the total microporous membrane system presented higher mean for stomata density on the abaxial face. Seedlings cultivated under microporous membrane containing systems showed greater development and organization of vascular bundles. According to the results obtained in this research, the sucrose suppression in the culture medium associated with the use of microporous membranes allows to obtain plants with better morphophysiological quality, besides reducing the production costs.

Keywords: seal, sucrose, microporous membrane, chlorophylls, stomata

1. Introduction
In vitro plant cultivation consists of different techniques that have advantages over conventional cultivation. The success of these techniques requires specific knowledge about the influence of physical and chemical factors on the regeneration and development of propagules. However, when it comes to large-scale production, production costs can be high [20]. Conventionally, *in vitro* cultivation of plants is carried out using exogenous supplementation with a main source of carbon (usually sucrose), to maintain the growth and development of the propagules, since the concentration of CO_2 inside the containers of cultivation is quite reduced, thus limiting photosynthesis [17, 11]. Also, the light supply is sometimes not suited to the photosynthetic needs of plants.
These factors alone or together can compromise the morphology and physiology of micropropagated plants [14]. Therefore, the intelligent use of physical and chemical factors increases the autotrophic ability of plants, allowing rapid adaptation to environmental conditions during acclimatization [54, 2].
Photoautotrophy is one of the main trends *in vitro* cultivation, being considered economically efficient since it reduces the chemical inputs used in the culture medium and significantly improves the quality of the seedlings that will be taken to the field [37, 51]. Environmental conditions in vitro heterotrophic cultivation is not appropriate, as they have high relative humidity, ethylene accumulation, low CO_2 concentrations and increased osmotic balance caused by the presence of sucrose in the culture medium [14].
The cultivation of plants in vitro with sucrose suppression is more advantageous since it reduces the risk of microbial contamination and production costs. Also, the addition of sucrose in the culture medium has an effect on the levels of photosynthetic pigments and on the anatomy of plants *in vitro* [14].
With the reduction or total suppression of sucrose in the culture medium, it is necessary to have a supply of CO_2 inside the culture vessels for photosynthesis. However, conventional sealing of cultivation containers limits the internal concentration of CO_2, hampering the photosynthetic ability of micropropagated plants [16]. Thus, the use of CO_2 permeable filters has benefits in ventilation inside the containers favoring the growth of propagules [37], in addition to reducing the occurrence of morphoanatomical disorders in micropropagated plants, such as hyperhydricity [39].
Photoautotrophic micropropagation can benefit the production of seedlings of several plant species, in particular, assist reforestation and environmental restoration programs with the photoautotrophic cultivation of tree species [48].
Although photoautotrophic cultivation is advantageous and is being used in different species [1], there are still few studies using this technique in vitro cultivation of native tree species of ecological and socioeconomic interest [21, 36, 38, 43,

[54]. In this sense, one of the native tree species of great importance for the Brazilian industry is *Myracrodruon urundeuva* Allemão [12]. It is a tree species of great economic value due to the chemical properties of the bark, which is widely used in the treatment of various diseases [3, 31, 42]. Also, its wood is widely used in the construction industry, standing out for being practically imputrescible [15]. In ethnobotanical studies for the conservation of plant diversity in Caatinga areas, the impact caused by the use of native forest on the conservation of species was verified. For this reason, *M. urundeuva* was included in the group of species with priority for local conservation in Northeast Brazil [30].

In this sense, the present research aimed to evaluate the effect of different sucrose concentrations and seal types on the growth and morphophysiological quality of *M. urundeuva in vitro*, to reduce production costs and improve the quality of micropropagated seedlings. Considering the following hypotheses: 1) The reduction or total suppression of sucrose in the culture medium promotes the reduction of plant growth and development, in addition to the appearance of morphophysiological disorders; 2) The use of microporous membranes can increase the content of photosynthetic pigments and improve the quality of plants in vitro regardless of the presence of sucrose in the culture medium.

2. Material and methods

The experiment was carried out at the Laboratory of Applied Biofactory Research of the Center for Strategic Technologies of the Northeast (CETENE), located in Recife /PE-Brazil. Diaspores of *Myracrodruon urundeuva* Allemão were obtained from the IPA José Nilson de Melo Experimental Station (Agronomic Institute of Pernambuco) in the municipality of Caruaru (PE), under the geographic coordinates 8° 14 'and 35° 55' in December 2015.

Diaspores were used as explants, which were disinfected in alcohol (70%) for one minute and subsequently in sodium hypochlorite (2.5%) for 15 minutes. The explants were then inoculated into test tubes containing 10 ml Woody Plant Medium (WPM) [28] at 50% salt concentration plus 0.1 g. L^{-1} inositol and 5.5 g. L^{-1} agar. Three sucrose concentrations (0.0, 7.5 and 15.0 g. L^{-1}) combined with three seal types were tested: total with cap (TS), total with 3M® microporous membrane (TSM) and partial seal (PS), where the latter consisted of a 5mm^2 opening foil lid sealed with a 3M® microporous membrane. After inoculation, the explants were kept in a growth room at 25 ± 2 °C in the dark. After emergence, the seedlings were transferred to shelves with white LED lamps, with a light intensity of 42 µmol m^2 s^{-1}. The seedlings were kept under these conditions for 45 days.

To obtain the CO_2 concentration, five empty test tubes were capped with their seals (TS, TSM, and PS) and kept in a growth room under the same environmental conditions as the tubes containing the mastic diaspores. Measurements of CO_2 concentration were performed weekly with the Vaisala meter model MI70.

Evaluations were performed at 45 days of cultivation. The experimental design was completely randomized in a 3x3 factorial arrangement (three sucrose concentrations and three sealing types) with 24 replications per treatment.

2.1 Growth Parameters

Fresh weight (FW), seedling height (H), number of leaves/plant (NL) (transformed data $\sqrt{x+1}$) and leaf area were evaluated.

2.2. Biochemical Analyzes

Chlorophyll and total carotenoid contents were determined from the maceration of 0.1 g of fresh leaves in acetone (80%), completing the volume to 12.5 ml. The obtained extract was filtered through fine mesh nylon mesh and then centrifuged for 5 minutes at 2,000 *g*. Spectrophotometric readings for chlorophyll content determination were taken at 645 and 663 nm [25] and carotenoids readings were taken at 470 nm [26]. Results were expressed in mg.g $^{-1}$FW.

2.3. Morfoanatomic evaluations

For the analysis of the internal morphology of the *M. urundeuva* leaves, segments of the median leafs region were fixed in 70% FAA solution [49]. Subsequently, the samples were dehydrated in an ethyl series [18], infiltrated and embedded in paraffin. The cuts were made at 8 µm in a Zeiss rotary manual microtome, model HYRAX M55. The sections were stained with safranin (1%) and alcian blue (1%) solution [4] and mounted on Entellan®. Then the slides were photographed in a Zeiss Axio ZL Apotome fluorescence microscope.

For scanning electron microscope evaluations, leaf segments of approximately 5 mm^2 were fixed in Karnovsky's solution [19]. Subsequently, the segments were rinsed three times with cacodylate buffer (sodium cacodylhydrate, CO_2, Sigma Aldrich, St. Louis, USA), dehydrated in an ethyl series to a critical point using the Bal-Tec CPD 030, Bal-Tec, Balzers, Liechtenstein, Germany. Then the segments were metalized with fine gold (Denton Desk II Sputter Coated Metallizer, Torontech Group International Markham, ON, Canada). Images of the adaxial and abaxial leaf epidermis were obtained using a scanning electron microscope (SEM), Quanta 200F model, Fei. Assessments of the density of trichomes, stomata, as well as the stomatal perimeter, central vein, and secretory ducts, were performed with the support of ImageJ software [50].

2.4. Statistical analysis

Data were subjected to analysis of variance and means compared by Tukey test at 5% probability. For statistical analysis, the Sisvar Software [10] was used.

3. Results

The concentration of CO_2 within the test tubes varied depending on the type of seal (Fig. 1). In the second and third weeks, the tubes sealed with the TSM system presented the highest CO_2 concentrations (860 and 848 ppm for the second and third weeks, respectively). CO_2 concentrations did not differ between TSM and PS treatments in the second to last week. At the end of the experimental period, the internal CO_2 concentrations were reduced in all sealing treatments. There was no effect of the association of factors of sucrose concentration and seal types for the variables FW, H, and NL. The use of the microporous membrane total sealing system (TSM) favored a 50% increase in seedling fresh weight (FW) compared to those under total sealing (TS), and 42% compared to those under partial sealing (PS) (Table 1).

The addition of sucrose in the culture medium did not influence the seedling's FW, and the possibility of sucrose suppression in the micropropagation of this species could be considered (Table 1). The seedlings presented higher height (H) when cultivated in microporous membrane sealed tubes, especially the TSM, with an increase of more than 60% compared to the TS system and 22.7% when compared to the PS. The culture medium without sucrose favored the best average seedling LA values (Table 1). The use of microporous membrane containing sealing systems (TSM and PS) positively influenced the increase of leaf number (NL) *in vitro* cultivated *M. urundeuva* seedlings. The addition of 15.0 $g.L^{-1}$ sucrose in the culture medium stimulated leaf emission, promoting an increase of 12% about the treatment with suppression of this component (Table 1). The effect was observed on the interaction of factors for the leaf area (LA) variable according to ANOVA. Under the TS system, the seedlings showed higher LA when they were cultivated with 7.5 or 15.0 $g.L^{-1}$ sucrose (Fig. 2f).

In the TSM system, sucrose concentrations did not influence LA. However, in PS the seedlings cultivated with 15.0 $g.L^{-1}$ sucrose presented a higher average for this variable. Seedlings cultivated under sealing systems containing microporous membrane had higher averages for LA, with more than 185% increase in seedlings cultivated under TSM compared to the TS system. Seedlings cultivated in the medium without sucrose reduced the number of leaves to invest in leaf area (Table 1; Fig. 2f). When sealing systems were compared within each sucrose concentration, TSM was the system that most stimulated leaf growth and expansion at all sucrose concentrations (Table 1 and Fig. 2f).

For biochemical evaluations, ANOVA detected an influence on the association of factors *in vitro* culture of *M. urundeuva*. In seedlings cultivated in medium without sucrose, the highest accumulation of Chl was observed in PS treatment (25.0 $mg.g^{-1}FW$), followed by TSM (22.2 $mg.g^{-1}FW$). The contents of Chl a were also higher in sealing systems containing microporous membrane, with emphasis on PS when combined with 7.0 and 15.0 $g.L^{-1}$ sucrose, reaching mean of the 31.2 and 32.1 $mg.g^{-1}FW$, respectively (Fig. 2a).

In the culture without sucrose addition, the PS system favored the highest increase in Chl b (9.8 $mg.g^{-1}$ FW). The addition of 7.5 $g.L^{-1}$ sucrose in the culture medium favored the increase of this pigment content in seedlings cultivated under microporous membrane systems without presenting the statistical difference between PS (14.7 $mg.g^{-1}$ FW) and TSM (13, 4 $mg.g^{-1}$ FW). In the medium containing 15.0 $g.L^{-1}$ sucrose, PS treatment promoted higher accumulation of chl b (22.0 $mg.g^{-1}$ FW), followed by TSM (16.4 $mg.g^{-1}$ FW) (Fig. 2b). Both partial sealing and increased sucrose concentration promoted higher accumulations of chl b.

Only seedlings cultivated in sucrose-containing media showed an increase in Chl t content, especially in the PS system, with mean values of 46.0 $mg.g^{-1}$ FW in medium containing 7.5 $g.L^{-1}$ sucrose and 51.6 $mg.g^{-1}FW$ in the addition of 15.0 $g.L^{-1}$ sucrose (Fig. 2c).

The sucrose suppression in the culture medium favored the increase in CAR contents in seedlings cultivated under the TSM system. When 7.5 $g.L^{-1}$ sucrose was added, seedlings cultivated under PS and TSM systems showed increased pigment content with an increase of 57.2% for PS compared to TSM. The increase of 15.0 $g.L^{-1}$ of sucrose in the culture medium did not differ statistically about the sealing system. Tector trichome density (TTD) was influenced only by the sealing factor on the adaxial face (Fig. 3 and 4).

A similar result occurred concerning the abaxial face, where the density of the tector trichomes was influenced by the sealing system (Fig. 3 and 5).

Plants cultivated under microporous membrane-containing systems (TSM and PS) showed a higher density of tector trichomes per mm^2 on the adaxial surface (TTAD) (Fig. 3a). On the other hand, on the abaxial surface, seedlings cultivated under the VT system showed higher tector trichome density (TTAB) (Fig. 3b; Fig. 5a).

The analysis of variance did not detect a significant difference in the variable density of glandular trichomes of the abaxial face. While density of glandular trichomes on the adaxial surface (GTAD) was influenced by the interaction of factors evaluated only in the TS system, in which increments were observed as the sucrose concentration was increased. In the TSM and PS systems, there were no significant differences regarding sucrose concentrations (Fig. 3b).

According to ANOVA, stomata density on the adaxial surface (SDAD) was influenced by the interaction between sucrose concentrations and seal types. The TS system positively influenced the increase in SDAD when combined with 7.5 $g.L^{-1}$ sucrose (Fig. 6).

In the TSM system, seedlings cultivated without sucrose presented a higher average for stomata density on the abaxial face (SDAB). For PS, the highest mean value was observed when seedlings were cultivated in the medium added with 15.0 $g.L^{-1}$ sucrose (Fig. 6a).

There was a significant influence of the evaluated factors on SDAB (Fig. 6b). Seedlings cultivated under TS and TSM systems showed higher SDAB when grown in the medium with the addition of 15.0 $g.L^{-1}$ sucrose. In the PS system, higher mean values were obtained in seedlings grown in medium without sucrose.

No influence of sealing systems on the stomatal perimeter of the adaxial surface (SPAD) was observed. However, about sucrose concentrations, it was found that the largest stomatal perimeters of the adaxial face were found in seedlings grown between 0.0 and 7.5 $g.L^{-1}$ sucrose (Fig. 6c). The stomatal perimeter on the abaxial face (SPAB) was influenced only by the sealing factor, where the TS system presented the highest mean between treatments, with increases of 12.5 and 8.5% about the PS and TSM, respectively (Fig. 6d).

The leaves' central vein perimeter (CVP) was influenced by the isolated factors, obtaining higher averages when the seedlings were cultivated in microporous membrane (TSM and PS) systems. Sucrose concentration of 7.5 $g.L^{-1}$ was favorable to the development of the main leaf vein (Fig. 7a and 8 d-i).

In general, the seedlings with the highest CVP were those grown in medium with addition of 7.5 $g.L^{-1}$ sucrose and in those grown under the TSM and PS systems (Fig. 7a). It was observed that the seedlings cultivated in these systems presented a more developed vascular system and more organized xylem bundles and vessels (Fig. 8d-f). The results obtained in the morphophysiological analyzes support the responses in the parameters of growth and biochemistry, consolidating the indication of the combined TSM system to

the medium without sucrose addition to start *in vitro* cultivation of *M. urundeuva* from seeds.

The number of secretory ducts (NSD) varied as a function of the interaction between sucrose concentrations and seal types. The most prominent result for NSD was in plants grown under TSM with sucrose suppression in the culture medium (Fig.s 7b).

The perimeter of the secretory ducts (PSD) was larger when the seedlings were cultivated in a microporous membrane containing sealing systems (TSM and PS). Sucrose concentration did not influence PSD (Fig. 7c and 8d-i).

Mesophyll thickness (MT) was highly influenced by the sealing system and the sucrose concentration in the culture medium. Seedlings grown in TSM systems combined with medium containing 7.5 $g.L^{-1}$ sucrose showed leaves with the most developed mesophyll (Fig. 7d and 8e). On the other hand, seedlings grown under the TS system showed a reduction in MT when this sealing system was combined with 15 $g.L^{-1}$ sucrose.

There were no significant differences between sucrose concentrations in the PS system (Fig. 7d). In general, seedlings grown under the TSM system showed greater development of xylem vessels and bundles, as well as greater cell organization (Fig. 8d-i).

4. Discussion

Photosynthesis of plants grown *in vitro* is affected by several factors, including explant type, stage of development, CO_2 concentration, quality of light supplied and the exogenous presence of sucrose in the culture medium [7]. Conventional cultivation conditions are generally characterized by a sealing system that maintains high relative humidity, low CO_2 concentrations, and ethylene accumulation within the culture vessels [34]. Also, sucrose is one of the most expensive components used *in vitro* culture media [46], and when high concentrations of this component are added to the culture medium they can negatively influence plant growth and development [14].

Divergences in plant responses upon exposure to exogenous sucrose application are discussed, where some plants have improved growth in the presence of this component, while other species show growth retardation. These responses may reflect the multiple roles of sucrose in plant growth [44]. Also, one can consider as a factor for these differences, the particularity in the metabolism of each species.

Authors [24] found that carbohydrate metabolism dynamics *in vitro* cultivated plants with high sucrose concentrations did not present growth advantages during acclimatization. On the other hand, plants from low sucrose media photosynthesize more easily to sustain growth and development [29]. Authors [9] also observed reductions in photosynthetic pigment contents when there was a reduction of sucrose concentration in the culture medium to *Musa acuminata*. Besides that, *in vitro* cultivation of sucrose-suppressed plants, the maintenance of photosynthetic pigment contents is closely related to the ability of some species to improve their performance under these cultivation conditions [22, 33].

Authors [59] verified that the increase of sucrose concentration in the culture medium negatively influenced the root growth of *Trichosanthes kirilowii* Maxim shoots *in vitro*.

In vitro plant growth is also greatly influenced by the quality of light, and in this sense, is an important factor in photosynthetic pigment contents, as it provides light at a specific wavelength, increasing photosynthetic efficiency [6,56]. This may partly explain the slight decline in chlorophyll content found in the present study, since in the present research only one light source was used. Although chlorophyll content decreased in seedlings grown with sucrose suppression, the chlorophyll a / b ratio was higher in the TSM system without sucrose addition.

The increase in chlorophyll a/b ratio indicates a significant change in photosystem stoichiometry that may reflect the efficiency of the photosynthetic process [55]. This result is confirmed by the results obtained in the present research, when the fresh mass, height, and leaf area were evaluated (Table 1 and Fig. 1e). The increase in leaf area is closely related to the determination or estimation of the photosynthetically active surface [13, 57] as well as the size of the central rib, which plays a major role in hydraulic conditions and the ability to export photoassimilates [40]. Authors [16] reported that the increase in biomass incorporation in mixotrophic crops depends on the photosynthetic capacity of the species.

Among the pigments involved in the photosynthetic process, carotenoids (CAR) play an important role in protecting cells against oxidative damage caused by reactive oxygen species (ROS) [15, 41]. The increase in CAR (Fig. 2d) contents verified in seedlings cultivated with microporous membrane and in the absence of sucrose favors the protection of photosynthetic apparatus, favoring photosynthesis and consequently plant growth [47, 52].

Inhibition of the photosynthetic process by exogenous sucrose supply is also related, among other factors, to the decrease in photosynthesis-related enzyme activity, reduction in quantum efficiency of photosystem II and reduction in stomatal conductance [29], where the stomatal conductance (stomatal opening and closing control) is responsible for controlling gas exchange between the interior of the culture vessel and the external environment, and regulating this gas flow is essential to meet the mesophyll demand for CO_2 [23].

As a consequence of the conventional sealing system not allowing good gas exchange, the stomata of *in vitro* grown plants are generally poorly functional, which can affect photosynthesis and consequently the growth of micropropagated plants (Table 1) [1, 17, 32]. Stomach density, morphology and size are important variables that may determine the speed of stomatal responses, reflecting on plant yield [23]. It has been proposed that for some species, smaller stomata exhibit faster stomatal movement because they allow for faster changes in solutes and control of stomatal conductance relative to larger guard cells [8], which may justify changes found in the abaxial stomatal perimeter of *M. urundeuva* seedlings grown under microporous membrane (TSM and PS) systems (Fig. 6d). Partial sealing systems using filters and microporous membranes provide increased gas exchange, directly influencing the structural dynamics of the leaf and the physiological characteristics of stomata and trichomes (Fig. 3, 4, 5 and 6).

Trichomes are associated with the protection of plants against adverse environmental conditions, reducing water loss through transpiration, reflecting excess light to protect the photosynthetic apparatus, as well as the production and storage of lipophilic compounds with pathogen protection function [27, 45, 58]. Therefore, increased density of tector and

glandular trichomes is an interesting feature to be considered in plants from *in vitro* cultivation (Fig. 5).

Different from what was found in the present research, authors others [53] found that sucrose suppression in the culture medium negatively influenced promoting cell disorganization, altering the internal morphology of *Pfaffia glomerata* leaves.

Although some studies report the importance of sucrose culture media supplementation [14, 35] this research confirms that for *M. urundeuva* is possible to establish protocol for mixotrophic micropropagation, with sucrose suppression in the medium cultivation and obtaining plants with better morphophysiological quality.

The morphophysiological and growth parameters indicate that sucrose suppression did not negatively influence the quality of the evaluated shoots in the present study. Thus, it was possible to establish an in vitro photomixotrophic cultivation protocol for *M. urundeuva*, where in addition to reducing costs it was possible to obtain plants with better morphophysiological quality for use in reforestation programs.

Finally, the understanding of the morphophysiological and biochemical responses of *M. urundeuva* in conditions of photomyxotropic cultivation enabled the establishment of an unprecedented protocol for this species that will support future studies in several areas, especially studies aimed at the sustainable use of this species, since it is priority species for local conservation.

Table 1: Fresh weight (FW), height (H), leaf area (LA) and the number of leaves (NL) of *Myracrodruon urundeuva* Allemão seedlings cultivated *in vitro* in culture media with different sucrose concentrations, associated with different types. Tube seal (TS = fully seal; TSM = fully membrane seal and PS = partial seal).

	Seal			Sucrose (g.L^{-1})			
	TS	**TSM**	**PS**	**0,0**	**7,5**	**15,0**	**CV %**
FW (g)	0.18 b	0.27 a	0.19 b	0.21 a	0.20 a	0.23 a	22.5
H (cm)	4.34 c	6.98 a	5.69 b	6.22 a	5.00 b	5.77 ab	17.1
NL	4.50 b	5.42 a	5.25 a	4.67 b	5.17 ab	5.33 a	10.7

Means followed by the same letter on the line do not differ statistically for each variable by Tukey a $p \leq 0,05$.

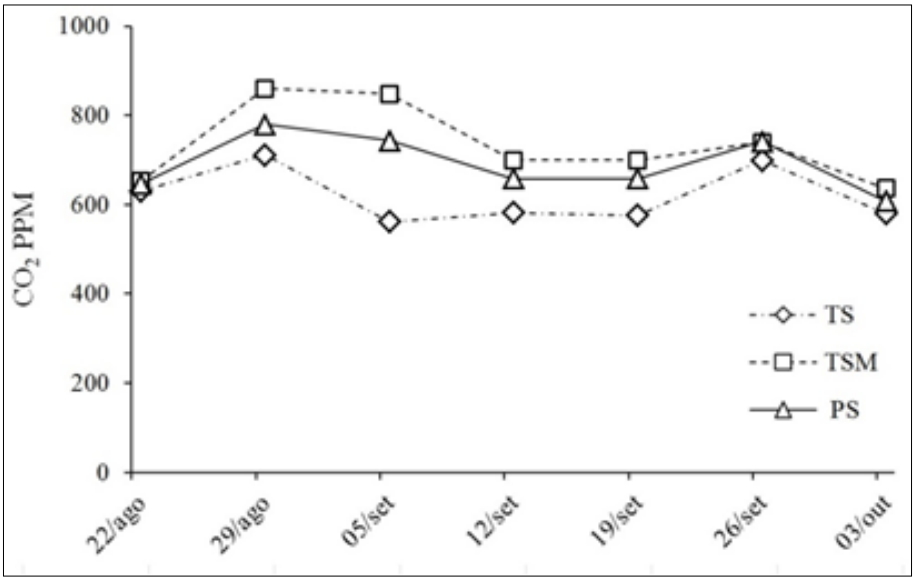

Fig 1: Concentration of CO_2 measured weekly during the experimental period. Total seal (TS), total membrane seal (TSM) and partial seal (PS).

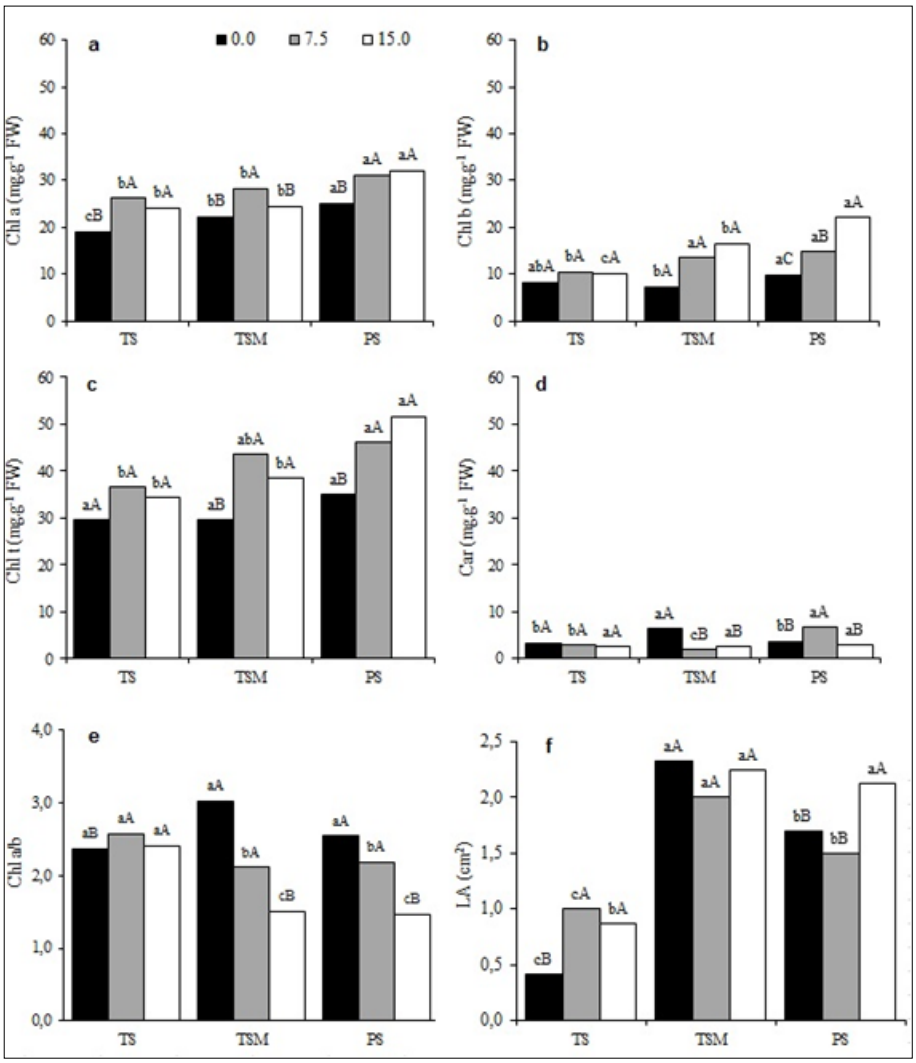

Fig 2: Leaf pigments content (a) chlorophyll *a*; (b) chlorophyll *b*; (c) chlorophyll *total*; (d) carotenoids; (e) chlorophyll *a/b* ratio and (f) leaf area *in vitro* cultivated *Myracrodruon urundeuva* Allemão seedling in culture media with different sucrose concentrations (0.0; 7.5; 15.0 $g.L^{-1}$) in association with different types of test tube seals (TS = total seal; TSM = total seal with membrane and PS = partial seal). Bars followed by same small letters for sealing types and uppercase letters for sucrose concentrations do not differ statistically by Tukey test $p \leq 0.05$.

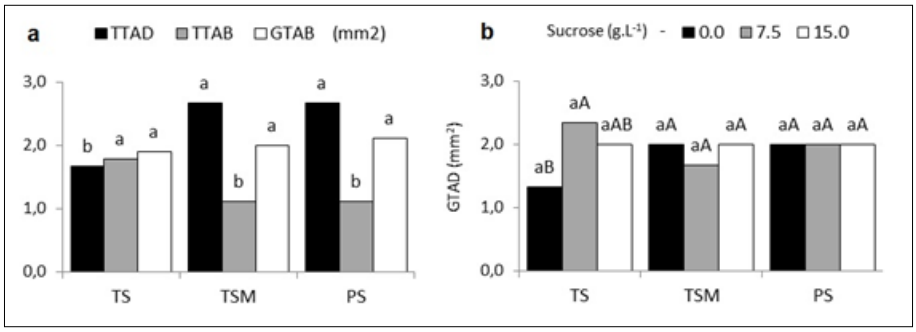

Fig 3: (a) Tectorial trichome density on adaxial (TTAD) and abaxial (TTAB) surfaces, glandular trichome density on abaxial (GTAB) and (b) abaxial (GTAD) surfaces in leaves of the *Myracrodruon urundeuva* Allemão cultured *in vitro* with different sucrose concentrations in combination with different types of test tube seals (TS = total seal; TSM = total seal with membrane and PS = partial seal). Bars followed by the same lower-case letters for sealing type and upper case for sucrose concentration did not differ statistically by Tukey test $p \leq 0.05$.

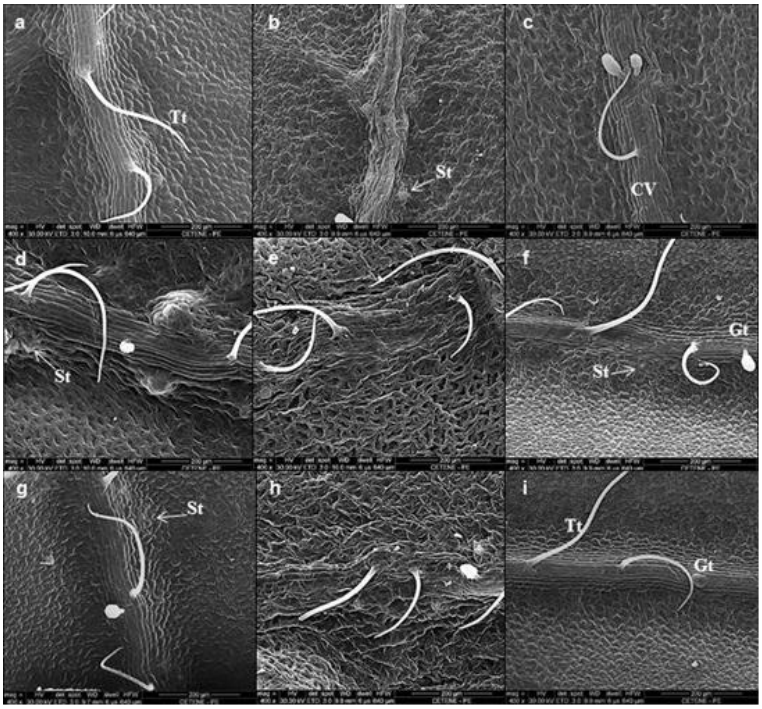

Fig 4: Scanning electron microscopy adaxial leaves of the *Myracrodruon urundeuva* leaves cultivated *in vitro* with different sucrose concentrations in combination with different types of test tube seals: (a = total seal and no addition of sucrose; b = total seal and addition of 7.5 $g.L^{-1}$ sucrose; c = total seal and addition of 15 $g.L^{-1}$ sucrose; d = total seal with membrane and no addition of sucrose; e = total seal with membrane and addition of 7.5 $g.L^{-1}$ sucrose; f = total seal with membrane and addition of 15 $g.L^{-1}$ sucrose; g = partial seal and no addition of sucrose; h = partial seal and addition of 7.5 $g.L^{-1}$ sucrose; i = partial seal and addition of 15 $g.L^{-1}$ sucrose. Legend: Tt = tector trichome; St = stomatal; CV = central vein; Gt = glandular trichome. (Bars =200 µm).

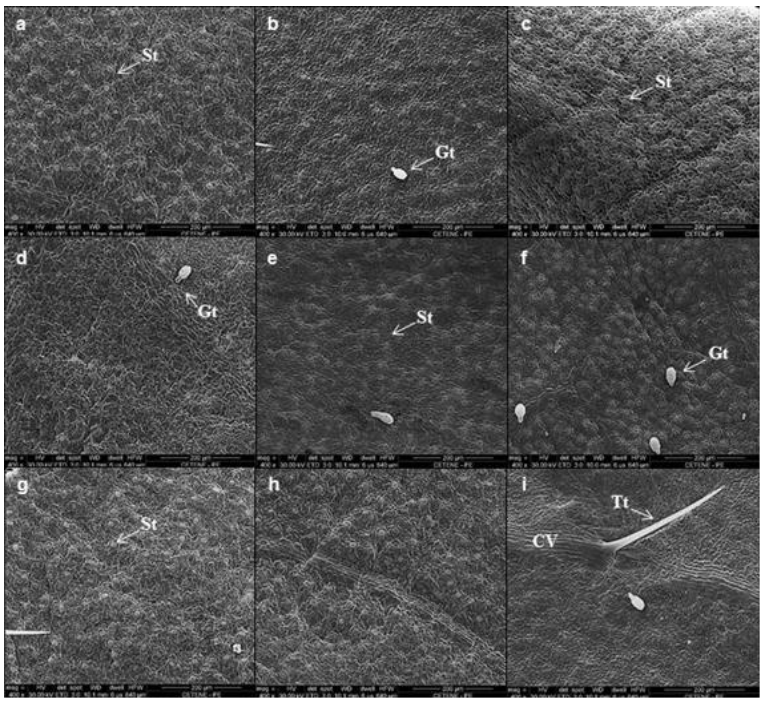

Fig 5: Scanning electron microscopy abaxial leaves of the *Myracrodruon urundeuva* leaves cultivated *in vitro* with different sucrose concentrations in combination with different types of test tube seals: (a = total seal and no addition of sucrose; b = total seal and addition of 7.5 $g.L^{-1}$ sucrose; c = total seal and addition of 15 $g.L^{-1}$ sucrose; d = total seal with membrane and no addition of sucrose; e = total seal with membrane and addition of 7.5 $g.L^{-1}$ sucrose; f = total seal with membrane and addition of 15 $g.L^{-1}$ sucrose; g = partial seal and no addition of sucrose; h = partial seal and addition of 7.5 $g.L^{-1}$ sucrose; i = partial seal and addition of 15 $g.L^{-1}$ sucrose. Legend: Tt = tector trichome; St = stomatal; CV = central vein; Gt = glandular trichome. (Bars =200 µm).

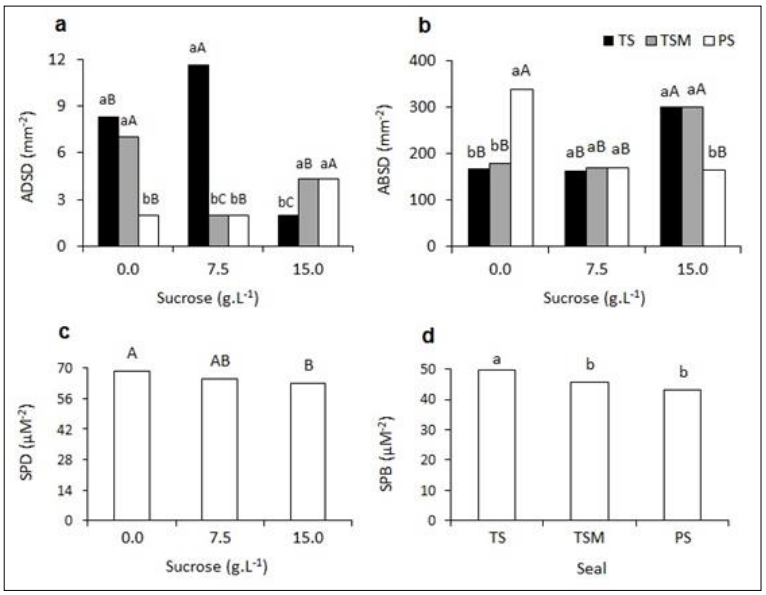

Fig 6: (a) Mean of Adaxial stomatal density (ADSD); (b) Abaxial stomatal density (ABSD); (c) stomatal perimeter on adaxial (SPD) and (d) stomatal perimeter abaxial surfaces (SPB) in leaves of the *Myracrodruon urundeuva* Allemão cultured *in vitro* with different sucrose concentrations in combination with different types of test tube seals (TS = total seal; TSM = total seal with membrane and PS = partial seal). Bars followed by the same lower-case letters for sealing type and upper case for sucrose concentration dis nor differ statistically by Tukey test p>0.05.

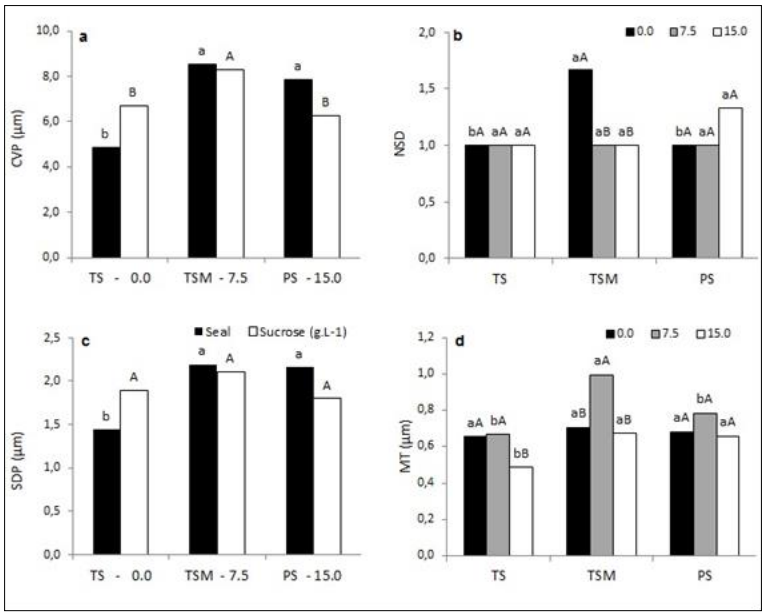

Fig 7: (a) Central vein perimeter (CVP); (b) number of secretory ducts (NSD); (c) secretory duct perimeter (SDP); (d) mesophyll thickness (MT) in leaves of *Myracrodruon urundeuva* Allemão cultured *in vitro* with different sucrose concentrations in combination with different types of test tube seals (TS = total seal; TSM = total seal with membrane and PS = partial seal). Bars followed by the same lower-case letters for sealing type and upper case for sucrose concentration dis nor differ statistically by Tukey test p>0.05.

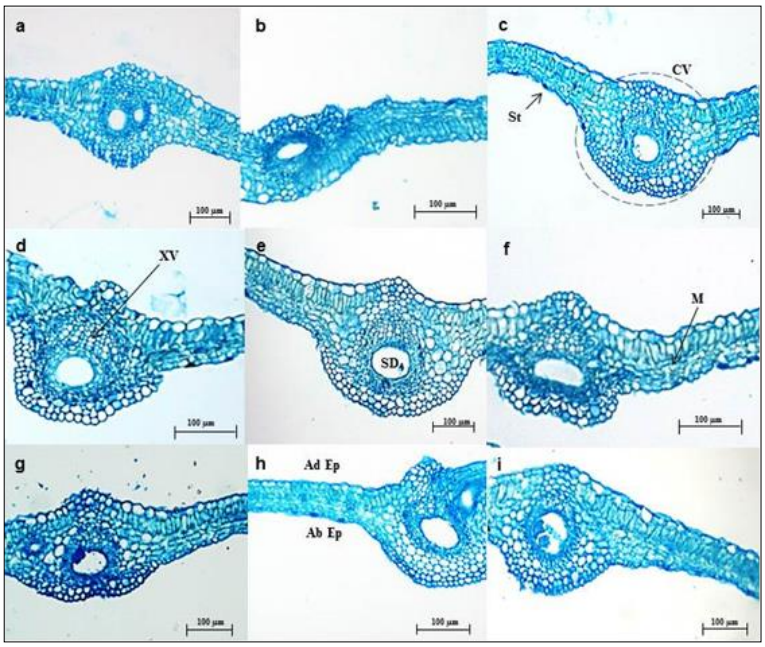

Fig 8: Cross-sectional microphotographs leaves of the *Myracrodruon urundeuva* leaves grown *in vitro* with different sucrose concentrations in combination with different types of test tube seals: (a = total seal and no addition of sucrose; b = total seal and addition of 7.5 $g.L^{-1}$ sucrose; c = total seal and addition of 15 $g.L^{-1}$ sucrose; d = total seal with membrane and no addition of sucrose; e = total seal with membrane and addition of 7.5 $g.L^{-1}$ sucrose; f = total seal with membrane and addition of 15 $g.L^{-1}$ sucrose; g = partial seal and no addition of sucrose; h = partial seal and addition of 7.5 $g.L^{-1}$ sucrose; i = partial seal and addition of 15 $g.L^{-1}$ sucrose. Legend: St = stomatal; CV= central vein; XV = xylem vessels; SD = secretory ducts; M = mesophyll; Ad Ep = adaxial epidermis; Ab Ep = abaxial epidermis.

5. Conclusions

Contrary to the initial hypothesis, the total suppression of sucrose in the culture medium promoted the growth and development of *M. urundeuva* seedlings *in vitro*, and improved characteristics of the internal morphology of the leaves.

The use of microporous membranes associated with total sucrose suppression improved the chlorophyll a/b rate, indicating a better photosynthetic performance, in addition to positively influencing seedling growth and development *in vitro*.

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