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Physio-anatomic aspects on the initial growth of *Guazuma ulmifolia* Lam. seedlings (Sterculiaceae)

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ABSTRACT

This paper aimed to evaluate the initial growth of “mutambo” seedlings in different conditions of light intensity and treatments with gibberellic acid (GA). The seedlings were kept under full sun and 50% of shading. Sixty days after the emergence, seedlings were sprayed with: 1) 100 mg L^{-1} GA_3; 2) 200 mg L^{-1} GA_3; 3) control. At the end of the appraisals, seedlings height under 50% of shading was compared to the height that were growing under full sun with 200 mg L^{-1} GA. Stem diameter was lower under shading. Leaf area did not vary among the treatments, but the root system growth was higher under full sun and did not vary among GA levels. The number of stomata, trichomes and epidermal cells on adaxial and abaxial sides was higher under full sun. Total dry masses of leaf and root were higher under full sun and with 200 mg L^{-1} GA application. “Mutambo” seedlings presented a higher initial growth under full sun, although with a lower height, diameter, and lenght of the largest root and total dry masses of leaf and root were higher. A concentration with 200 mg L^{-1} promoted a higher growth.

Key words: ecophysiology, gibberellin, mutambo.

INTRODUCTION

The use of native tree species in reforestation programs with sustainable management or for urban plantation has been intensified lately. For a rational exploitation of the potentialities of native species and for the recovery of areas with some type of damage, it is extremely important to study the auto-ecology of the species and the seedlings production (Lemos Filho and Duarte 2001, Almeida et al. 2004).

Papers about the use of phytoregulators in tree and shrub species are rare. According to Stefanini et al. (2002), gibberellin effects on seedlings can be noticed on stem elongation, internode lenght, leaf area and growth of dry mass. These effects promote cell division and elongation and, consequently, the plant increases cell wall extension and raises its strength due to the transversal orientation of cellulose microfibrils, which act mainly on young and meristematic cells however, species react differently to certain kinds of gibberellin (Kerbauy 2004).

Besides hormonal control, light plays an important role in the development of forest species. These need some luminous requirements for their development and the seedlings can use light to better develop in places with a high light intensity as in big glades, while other species have a better development in shading under woods. Seedlings of *Clitoria fairchildiana* R.A. Howard (“sombreiro”) presented higher height and bigger root dry mass under full sun, while in *Peltophorum dubium* (Sprengel) Taubert (“canafístula”) a significant difference for root height and length was not noticed, although it presented bigger root dry mass un-

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der full sun (Portela et al. 2001). However, seedlings of *Goupia glabra* Aulb. ("cupiúba") cannot be grown under full sun, because they may die. Meanwhile, all the growth characteristics increase with shading (Daniel et al. 1994).

According to Bjorkman (1981), leaf structure may be greatly influenced by the light level during the leaf growth, and it may change its structure by different levels of light, which is a common characteristic of species that present a large potential of acclimatization. Plant adaptation to the light environment depends on the adjustment of the plant to its photosynthetic apparatus. So, the environmental light can be used more efficiently as possible. Therefore, the growth efficiency may be related to the capacity of seedlings to adapt to the conditions of environmental light intensity (Santiago et al. 2001).

Lima Jr. et al. (2006), working with *Cupania vernalis* Camb., ("camboatã") demonstrated that the modifications of leaf anatomy may influence in the gases exchange rates and, so, promote great differences in the efficiency of light use (Schlüter et al. 2003), which shows that there is an adaptative plasticity in species that present a large potential of acclimatization (Nakazono et al. 2001, Ivanova and P'Yankov 2002, Piel et al. 2002, Schlüter et al. 2003).

Guazuma ulmifolia Lam., a plant of Sterculiaceae family commonly known as "mutambo", is a plant of arboreous size, semideciduous and a pionner species. It may reach ten meters in height and presents a large and irregular distribution. It is used as an ornamental plant in several tropical countries and has a good performance in the recovery of damaged areas, besides its medicinal use for the treatment of respiratory illnesses. Its seeds produce aromatic oils that are used by cosmetic industry and, in some countries, it is used as forage for cattle (Almeida et al. 1998, Durigan et al. 2002).

Few information was found in the inquired bibliography about leaf anatomy and seedlings production of "mutambo", so this paper aimed to evaluate the initial growth of its seedlings under different conditions of light intensity and the results of gibberellin application on its leaf anatomy.

MATERIALS AND METHODS

Seeds of *G. ulmifolia* were picked up from trees in Dourados, MS. Some dried ones were kept in a herbarium in Dourados under the number 2149. The sowing was carried out on trays with 128 cells in plantmax substratum, and 15 days after germination the seedlings were transplanted to plastic packings with 30 $\times$ 12 cm, containing soil + sand + manure (2:2:1), and kept under 50% of shading for 7 days. Afterwards, they were put under 50% of shading and under full sun in Horto de Plantas Medicinais at UFGD, in Dourados – MS. At the 60^{th} day after the emergence, the seedlings were sprayed with: 1) 100 mg L^{-1} of gibberellic acid; 2) 200 mg L^{-1} of gibberellic acid; and 3) seedlings with no treatment were used as control.

Initial Growth of Seedlings

Height and stem diameter were evaluated fortnightly and, at the 120^{th} day after the emergence, the length of the largest root, leaf area using LI-COR 3000, leaf dry mass, root dry mass, shoot dry mass and total dry mass, specific leaf area and leaf weight ratio were also evaluated (Benincasa 1988).

Experimental design was completely randomized in 2 (light levels) $\times$ 3 (gibberellin treatments) $\times$ 5 (seedling age) factorial arrangement, with 4 repetitions of ten seedlings each. Data were analyzed by F test and the means were compared by Tukey test at 1% of probability. It was also used the regression analysis to evaluate height and diameter data.

Study of Epidermal Appendices

To observe the trichome type, ten fully-expanded leaves of the seedlings of all treatments after 120 days were cut by free-hand using a steel blade in the median region of the leaf. The obtained cross sections were clarified with hypochlorite of sodium at 20% and after this, they were washed with acetic water 2%. They were also submitted to a dual colouration with astra blue and safranine (Bukatsch 1972), arranged in glycerinated jelly (Dop and Gautié 1928) and polished according to usual techniques described by Kraus and Arduin (1997).

Some crossed cuts were also made by hand with a steel blade in the median region of the leaf on the adaxial and abaxial surfaces. Besides cross sections and to analyze the epidermis, stomata types and the shape of ordinary epidermal cells, some prints with an instant tape (Super Bonder®) were made by sampling at random the leaves surface. It was used the dissociation technique by the crushing method from Jeffrey (Johansen 1940) until the complete dissociation of the epidermis, using aqueous safranine for colouring. The description and counting of epidermal cells, trichomes and stomata were conducted. Stomata counting was made with a Colleman microscope and the assistance of a bright camera, according to the methodology described by Labouriau et al. (1961) in three fields in the median region of ten leaves from ten distinct plants, totalizing thirty fields for each treatment.

According to a formula proposed by Wilkinson (1979), the stomatal index (Si) was calculated by:

$$Si\,(\%) = \left[\frac{Sn}{(Sn + En)}\right] \times 100,$$

where Si is the stomatal index, Sn the stomata number and En the epidermal cells number.

The data were analyzed by F test and, when there was a significant difference, the means were compared by Tukey test at 5% of significance.

The obtained results were recorded through drawings with the assistance of a bright camera adapted to a Coleman microscope, and with photographs of the entire plant using a Nikon Coolpix 4300. After the analysis of the slides, photomicrographs were produced in a trinocular microscope Labomed CXRII with a connected camera Sony Cyber Shot 4.1.

RESULTS AND DISCUSSION

Initial Growth of Seedlings

A significant interaction among the treatments was not observed for any of the evaluated characteristics. The growth in height under the condition of 50% of shading was bigger when compared to that under full sun. At the end of the evaluations, the height under 50% of shading (average of 41.49 cm) was bigger when compared to that of plants growing under full sun (average of 34.99 cm). Regarding the gibberellin application, only the concentration of 200 mg L^{-1} provided a significant increase in seedlings height (Figs. 1a, b).

The height of plants under moderate conditions of shading was higher as a consequence of its quick metabolism, which increases leaf surface to supply the photoassimilations of light. The speed of the phenotype reaction and the increase of growth rates are good qualities which allows these species to compete for light in the middle of a herbaceous vegetation, and are in fast expansion, such as the one that is present in the glades of the forest (Grime 1982 quoted by Moraes Neto et al. 2001).

The stem diameter did not vary during the evaluations and treatments however, after 120 days the stem diameter was height for plants under full sun than for those under 50% of shading, and did not vary among GA concentrations. In the evaluated period, leaf area did not vary among the treatments, but the growth of the root system was higher under full sun and did not vary among the gibberellic levels (Table I).

TABLE I

Diameter, leaf area and lenght of the largest root of *Guazuma ulmifolia* seedlings 120 days after emergence due to light intensity and gibberellin application.

Gibberellin/ Light levels	Diameter (cm)	Leaf area (dm^2)	Lenght of the largest root (cm)
Control	6.39 a	506.25 a	30.85 a
GA 100 mg L^{-1}	6.52 a	511.27 a	31.89 a
GA 200 mg L^{-1}	6.97 a	578.08 a	32.65 a
full Sun	7.01 a *	527.74 a	33.21 a
50% of shade	6.24 b	535.99 a	30.38 b

Means followed by the same letter (in column) do not differ among themselves by *, by F test and Tukey test at 1% of significance.

These results are similar to those found by Moraes Neto et al. (2001) who while studying different levels of light intensity, observed higher values for height and leaf area under conditions of 40% of light for all studied species, such as *G. ulmifolia*, but a greater total dry weight was found in this condition.

Root and leaf total dry masses were higher under full sun and with 200 mg L^{-1} GA application, while shoot dry mass did not vary significantly (Table II). In this treatment, it must have had a higher production and a partitioning of photoassimilates for other parts of the plant. The cell expansion provided by gibberellic acid may triggered the production of new cell compounds, so that the growth in height could occur.

The gibberellic acid application may be justified with this paper since the results with other species also varied due to the used concentration of phytoregulators. Similar results were observed by Stefanini et al. (2002) who used doses at 0, 10, 20 and 50 mg L^{-1} of gib-

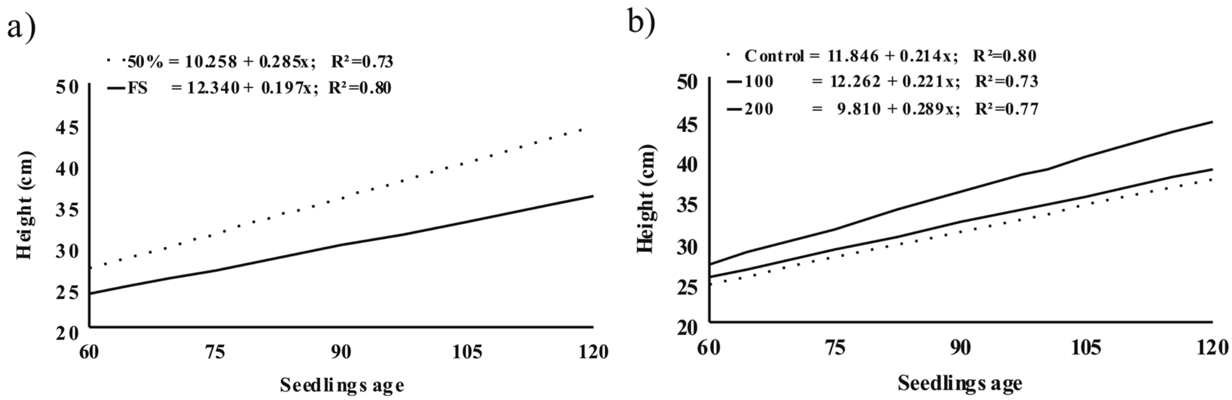


Fig. 1 – Height of *Guazuma ulmifolia* seedlings due to shading (a) and gibberellin doses (b).

TABLE II
Total dry mass of leaves and roots of *Guazuma ulmifolia* seedlings 120 days after emergence due to light intensity and gibberellin application.

Gibberellin/ Light levels	Total	Dry mass (g) Air space	Leaf	Root
Control	8.61 b	4.98 a	2.93 b	3.63 b
GA 100 mg L^{-1}	8.35 b	5.03 a	2.85 b	3.33 b
GA 200 mg L^{-1}	10.14 a	5.82 a	3.37 a	4.45 a
Full Sun	9.95 a *	5.54 a	3.45 a	4.41 a
50% of shade	8.12 b	5.01 a	2.65 b	3.19 b

Means followed by the same letter (in column) do not differ among themselves by *, by F test and Tukey test at 1% of significance.

berellic acid in *Lippia alba* (Mill.) N.E. Brown ("erva cidreira") and did not observe a significant effect of GA on the plants growth, their results justify the concentrations of phytoregulators used by them. Leaf weight ratio represents the capacity of photoassimilates translocation from shoot for all the other parts of the plant and the bigger this ratio the more efficient the translocation is, which favors the growth of all the other parts of the plant. During the evaluated period, any significant difference of this characteristic was not observed among the treatments. However, the specific leaf area was higher under 50% of shading (202.41 dm^2 g^{-1}). This is a coherent result considering that the seedlings under shading tend to present a higher leaf expansion, but with lesser thickness and, so, lesser leaf weight (0.33 g g^{-1}).

Epidermal Appendices

There was no significant interaction between light treatments and gibberellin applications for the number of epidermal cells on adaxial and abaxial surfaces, stomata and trichomes numbers (Table III). A significant difference on the trichomes number among the treatments with gibberellin was not observed either. However, under full sun, a higher number of trichomes was noted per unit/area, while the other characteristics were also bigger in seedlings under full sun and in those plants treated with 50 mg.L^{-1} of gibberellin.

In leaves of plants under full sun, the number of stomata was bigger, which differs statistically from the number of stomata kept under shading (Table III). Cormark and Gorham (1953) quoted that, on the abaxial surface, stomata are twice more numerous per unit/area in leaves under the sun than in those under shading. According to Voltan et al. (1992) and Morais et al. (2003), the increase of shading causes several anatomical modifications, such as the reduction in the stomata number, which is directly related to photosynthetic process. According to Lima Jr. et al. (2006), plants yielded in high levels of irradiance present a minor resistance

TABLE III
Stomata number on abaxial surface, cells number on adaxial and abaxial surfaces of *Guazuma ulmifolia* Lam.

Gibberellin/ Light levels	Stomata number on abaxial surface (mm^2)	Trichome number on abaxial surface	Number of epidermal cells on adaxial surface (mm^2)	Number of epidermal cells on abaxial surface (mm^2)
100 mg L^{-1}	47.6 a	0.250 a	100.40 a	148.60 a
200 mg L^{-1}	31.8 c	0.300 a	87.80 b	120.00 b
Control	41.2 b	0.400 a	79.00 b	129.60 ab
Full sun	41.4 a	0.533 a	92.80 a	142.53 a
50% of shade	36.0 b	0.100 b	85.33 b	122.93 b
CV (%)	17.9	17.4	13.4	22.4

Means followed by the same letter (small one in line and capital one in column) are equal among themselves by F* test and Tukey test at 5% of significance.

to gases diffusion, which reflects directly in the increase of photosynthesis.

The indicative factor of the adaptive plasticity of *G. ulmifolia* to different conditions of light refers to the significant increase of the number of ordinary epidermal cells on the abaxial surface under full sun when compared to the treatment at 50% (Table III).

Stomatal index was bigger for the control seedlings and it did not change among the light treatments. The increase in GA application under shading condition provided a reduction in the stomatal index (Table IV). However, in the seedlings under full sun, higher values were observed, but they did not vary significantly among the treatments with gibberellin.

From observations of the crossed sections of leaf blades, it was noticed that the number of stomata per area, the cells number and the stomatal index were higher in plants cultivated under full sun. These results agree with those of other species, in which an increase generally occurs in frequency and stomatal index with the irradiance elevation (Atroch et al. 2001, Hanba et al. 2002).

An increase in the stomatal density may allow the plant to raise the conductance of gases and, so, prevent the photosynthesis to the decrease under different environmental conditions. Some papers have demonstrated positive correlations between stomata number and photosynthetic rate (Castro 2002). Bjorkman and Holmgren (1963) comment that the stomatal frequency is a good signal of photosynthetic capacity, but the stomatal conductance depends on both stomata number and size.

The cross section on the abaxial surface of the leaves (Figs. 2, 4, 6, 8, 10 and 12) presents the epidermal cells generally with sinuous walls and more emphasized than on the adaxial surface (Figs. 3, 5, 7, 9 and 11), the stomata are mainly on the abaxial surface. On the adaxial surface the stomata were observed only next to the primary vein, being paracytic. On the abaxial surface, unicellular non-glandular trichomes, multicellular non-glandular and branched trichomes and glandular ones with or without stalk were also observed (Figs. 14 to 17).

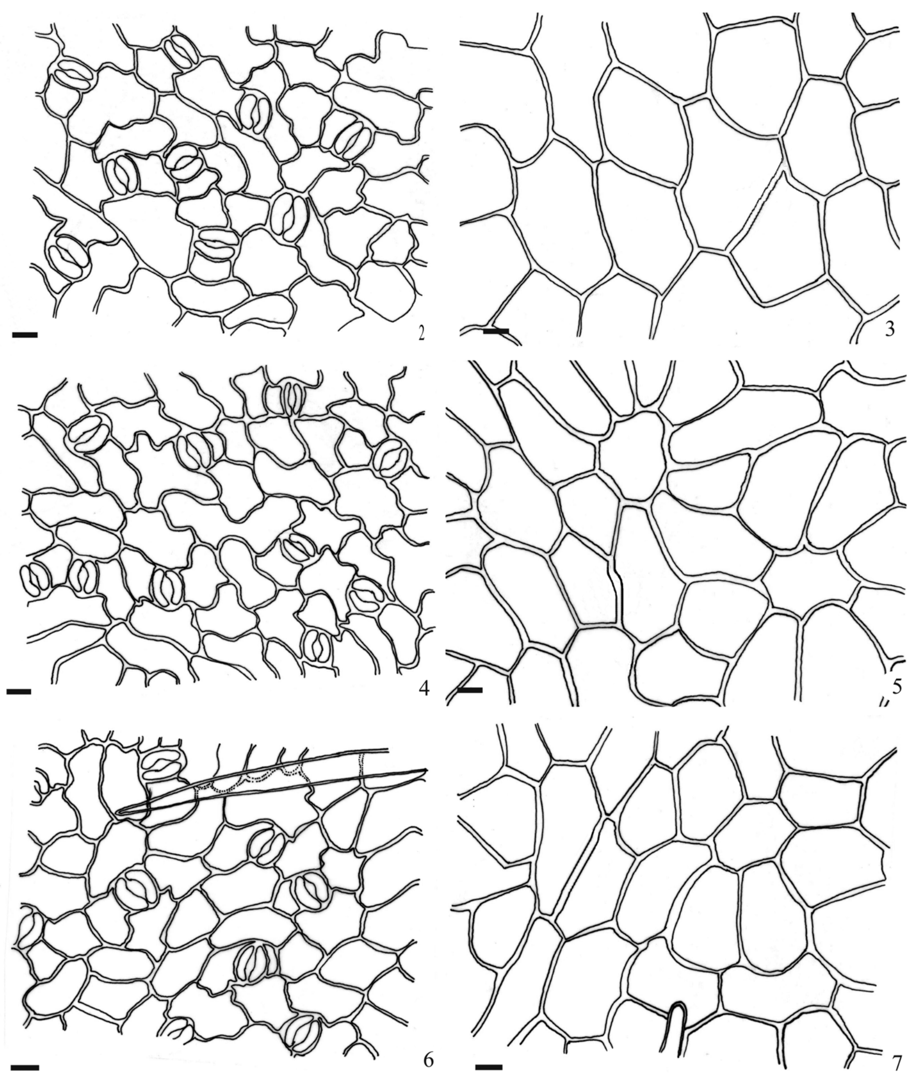
It was observed that the walls of the cells of plants yielded on shading were more sinuous. According to Santiago et al. (2001), the walls of the epidermal cells of *Piper hispidinervum* C. DC. ("pimenta longa") were thicker, straight on leaves under full sun and sinuous on leaves of a sub-forest, showing that environmental conditions, as solar radiation, influence on the growth and development of vegetable tissues. Studies carried out by Araújo and Mendonça (1998) with *Aldina heterophylla* Spruce ex Benth. ("Macacu De Paca") showed that the sinuosity of the wall of the epidermal cells is directly related to the exposition of plants under full sun or under shading, and that the thickening of the walls of these cells may be related to a high light intensity.

The present work determined that mutambo seedlings presented a better initial growth under full sun since all the evaluated characteristics presented higher values, except the height. Stomata, epidermal cells and

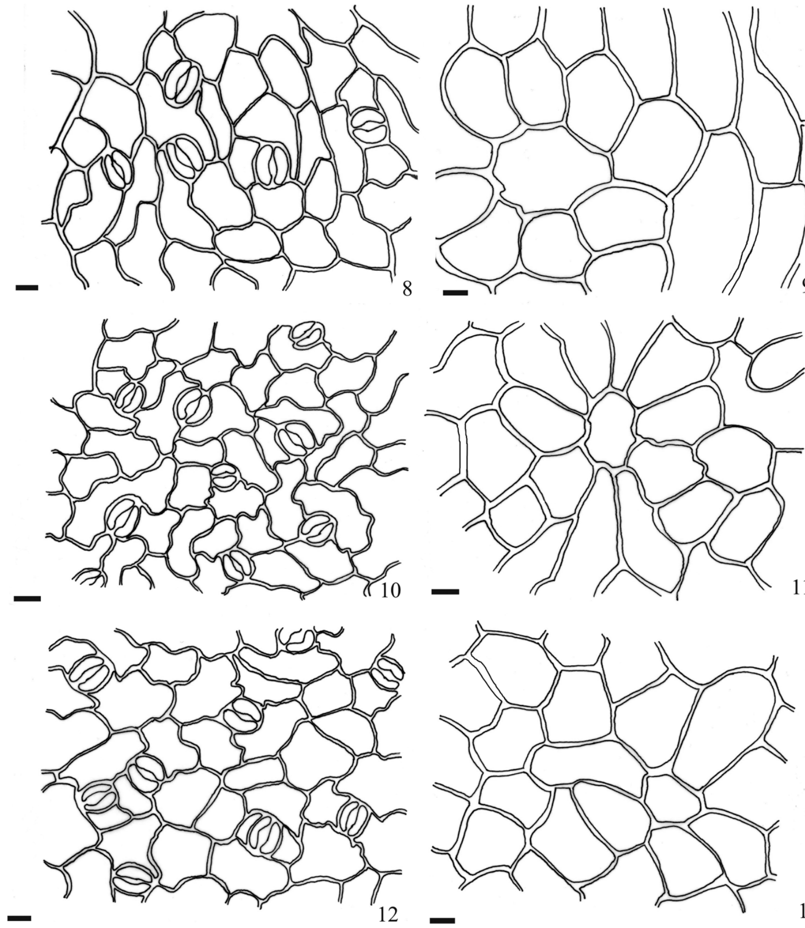
TABLE IV
Stomata index (%) on abaxial surface of the epidermis of "mutambo" seedlings (*Guazuma ulmifolia* Lam.).

Light level/	Stomatal index (%)		
Gibberellin	Control	GA100 mg L^{-1}	GA200 mg L^{-1}
50% of shade	18.9 Aa*	15.3 Bb	12.2 Bc
Full sun	17.8 Aa	17.9 Aa	17.4 Aa
CV= 15.8%			

Means followed by the same letter (small one in line and capital one in column) are equal among themselves by F* test and Tukey test at 5% of significance.



Figs. 2-7 – Diagrams of the crossed section of the epidermis of *Guazuma ulmifolia* Lam. at 50% of shading. 2. Abaxial surface of *G. ulmifolia* without gibberellin. 3. Adaxial surface of *G. ulmifolia* without gibberellin. 4. Abaxial surface of *G. ulmifolia* with gibberellin 100 mg L^{-1}. 5. Adaxial surface of *G. ulmifolia* with gibberellin with 100 mg L^{-1}. 6. Abaxial surface of *G. ulmifolia* with gibberellin 200 mg L^{-1}. 7. Adaxial surface of *G. ulmifolia* with gibberellin 200 mg L^{-1}. Bars = 50 μm.



Figs. 8-13 – Diagrams of cross section of the epidermis of *Guazuma ulmifolia* Lam. under full sun. 8. Abaxial surface of *G. ulmifolia* without gibberellin. 9. Adaxial surface of *G. ulmifolia* without gibberellin. 10. Abaxial surface of *G. ulmifolia* with gibberellin 100 mg L^{-1}. 11. Adaxial surface of *G. ulmifolia* with gibberellin with 100 mg L^{-1}. 12. Abaxial surface of *G. ulmifolia* with gibberellin 200 mg L^{-1}. 13. Adaxial surface of *G. ulmifolia* with gibberellin 200 mg L^{-1}. Bars = 50 μm.

trichomes numbers were also bigger under full sun. A concentration of gibberellic acid at 200 mg L^{-1} provided a higher increase in height and dry mass, although anatomical characteristics were bigger at 100 mg L^{-1} concentration.

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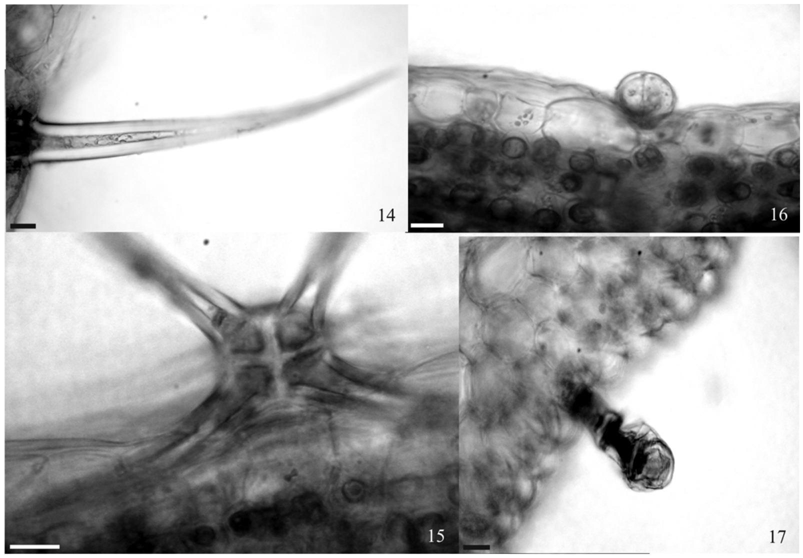
RESUMO

O objetivo desse trabalho foi avaliar o crescimento inicial das mudas de mutambo em diferentes condições de luminosidade e tratamentos com ácido giberélico (GA). As mudas foram mantidas em sombrite 50% de sombra e a pleno sol e aos 60 dias após a emergência as mudas foram pulverizadas com: 1) ácido giberélico 100 mg L^{-1}; 2) ácido giberélico 200 mg.L^{-1} e 3) testemunha. Ao final das avaliações a altura sob 50% de sombreamento foi maior comparada com aquelas crescendo a pleno sol com GA 200 mg L^{-1}. O diâmetro de colo foi menor sob sombreamento. A área foliar não variou entre os tratamentos, porém o crescimento do sistema radicular foi maior a pleno sol não variando entre os níveis de GA. O número de estômatos, tricomas e células epidérmicas nas faces adaxial e abaxial foram maiores a pleno sol. As massas secas



Figs. 14-17 – Trichomes of the metaphyll of *Guazuma ulmifolia* Lam. 14. Unicellular nonglandular trichome. 15. Multicellular nonglandular trichome, branched. 16-17. Glandular trichome. Bars = 50 μm.

total, de folha e raiz foi maior a pleno sol e com aplicação de GA 200 mg L^{-1}. As mudas de mutambo apresentaram um crescimento inicial melhor a pleno sol, embora com menor altura, o diâmetro, o comprimento da maior raiz e a massa seca total, de folha e de raiz foram maiores. A concentração de 200 mg L^{-1} de GA promoveu maior crescimento.

Palavras-chave: ecofisiologia, giberelina, mutambo.

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