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Propagation of *Aloysia citriodora* Palau Using Different Cutting Types under Light Intensity

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Authors' contributions

This work was carried out in collaboration among all authors. Authors OBS, AG and CCS in the planning, execution of the experiment, field data collection, statistical analysis, figure and table construction, data interpretation and scientific writing of the manuscript. Authors EPT, NAHZ and MCV provided resources and materials for the experiment, supervised the project, revised and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study was to evaluate the effect of types of cuttings and light intensity on cidró vegetative propagation.
Place and Duration of Study: Medicinal Plants Garden, Federal University of Grande Dourados (UFGD), Dourados, state of Mato Grosso do Sul, Brazil, September to November 2016.
Methodology: The experiment was carried out under two light conditions (shaded and full light), evaluating three types of cuttings (softwood, semi-hardwood and hardwood). Experimental design was a 2 x 3 factorial randomized complete block design (RCBD) with four replications.
Results: Hardwood cuttings showed the highest survival rates (86% and 82%), regardless of light. Highest fresh weight (0.6062 g $plant^{-1}$) and dry mass (0.2987 g $plant^{-1}$) with a leaf of 44.57 cm^2 were from hardwood cuttings, regardless of the light, while the longest root length were those of softwood cuttings under full light. Physiological indices varied as a function of light intensity, The

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highest values of leaf area ratio (46.11 cm^2 g^{-1}) and specific leaf mass (0.0037 g cm^{-2}) occurred in cuttings under full light.
Conclusion: The vegetative propagation of cidró can be carried out using the hardwood portion of the cutting branch, under full light.

Keywords: Cultural methods; medicinal plants; cuttings production; full light; shaded.

1. INTRODUCTION

Aloysia citriodora Palau (*Verbenaceae*, cidró) is native to South America, especially Argentina and Chile. It is grown in southern Brazil and other countries in ornamental and medicinal gardens [1]. The plant is a shrub, and its leaves are used in folk medicine as a mild sedative, against fevers, antispasmodic, digestive, stimulant, tonic, and soothing [2]. They have antioxidant, antibacterial [3], antispasmodic [4], sedative and cardiovascular [5] activities. The plant is also used in the *in vitro* control of *Fusarium* sp. [6], and essential oil as a repellent against *Aedes aegypti* L. [7]. The species has the potential of agronomic cultivation and high levels of secondary compounds [8]. Therefore, there is a need for information regarding the propagation of the species in order to obtain high-quality cuttings.

The propagation of medicinal plants by cuttings has become widespread, since it maintains the genetic characteristics, promoting the standardization of the raw material from the multiplication of clones with a high content of essential oil and significant constituent [9]. Besides, it is an alternative for species that have small seeds of delicate collection, low germination index due to dormancy and/or seed immaturity, as reported for *Verbenaceae* species [10].

The propagation process is influenced by many factors like the part of the cuttings to be collected, light luminosity and composite of substrate. This is because the amount of reserves in the plant tissue varies along the branch; thus, cuttings of different parts tend to differ regarding to rooting [11]. Studies of this nature may contribute to decision making at the time of material collection and propagation.

Another factor that can result in changes in the responses of cuttings establishment is the light intensity. The level of incident solar radiation may affect the physiological processes of cuttings and the early development of plants [12]. Literature indicates that cuttings propagated by *Ficus carica* L. [13] and *Cornus alba* L. [14] responded differently to shade levels but works with *A. citrodora* were not found, making it necessary to study and to investigate the light regimes in the cuttings production phase and the acclimatization processes.

Considering the medicinal importance, the advances in studies regarding bioactivity and the need for new information on propagation. The hypothesis for this study is that the light ambience and the branch portion of the cuttings may influence the morphophysiological characteristics of species and obtaining high quality cuttings. The aim was to evaluate the effect of cuttings type and light intensity on vegetative propagation by cidró.

2. MATERIALS AND METHODS

The experiment was carried out from September to November 2016, in the Medicinal Plants Garden (22º11'43.7"S and 54º56'08.5"W, 452 m above sea level) at the Federal University of Grande Dourados (UFGD), Dourados, state of Mato Grosso do Sul, Brazil. The climate is classified as Am (Tropical Monsoon Climate), with annual average temperature of 22 to 24 ºC and an annual rainfall of 1300 to 1600 mm [15].

The propagation of cidró was studied using three types of cuttings, softwood, semi-hardwood and hardwood, under two light intensity, shaded (polyethylene plastic covering + sombrite® 50%) and full light. Experimental design was a 2 x 3 factorial randomized complete block design (RCBD), with four replications and ten experimental units per plot. Transparent plastic containers (500 mL) were used per cutting unit. Example of plant material is deposited in the DDMS Herbarium - Dourados-MS, under number 5632.

Cidró cuttings were collected in the early hours of the morning of matrix plants from the garden of Escola Agrotécnica José Pereira Lins (22º10'33,06'' S and 54º41'24,32'' W, 347 m above sea level) in Dourados, state of Mato Grosso do Sul, Brazil. The cuttings had average dimensions of 15 cm length, average diameters (mm) of 1.40; 3.49; 4.09 and number of leaves of 10; 6; 0 for softwood, semi-hardwood and hardwood cuttings, respectively. After cutting, they were placed in a container with water to avoid dehydration and then planted in the containers filled with substrate base composed of 50% dystroferric Red Latosol (1:1, v/v) + 50% Tropstrato®. According to Silva [16] methodology, the chemical attributes of the mixture were, as follows: pH in $CaCl_2$ = 5.94; P (mg dm^{-3}) = 42.65; K; Ca; Mg; H + Al ($cmol_c$ dm^{-3}) = 1.99; 13.28; 19.00; 2.37, respectively; V (%) = 98.88. The soil physical attributes were total porosity = 61% and real density = 0.94 g cm^{-3}. Daily irrigations were performed over the experimental period to maintain 70% of the field capacity of the substrate, using sprinkler system and weeding of invasive plants.

At 60 days, the survival of the cuttings was evaluated, and three cuttings of each plot were harvested, removing them from the substrate (Fig. 1). Afterwards they were washed to remove the excess of substrate adhered to the roots, counting the buds, leaves, and lengths of buds and of the largest root, using a graduated ruler (cm). Next, the cuttings were separated into leaves, stems and roots and had their leaf and roots areas measured by using an area integrator (LI-COR, Model 3100C; Nebraska-USA). After weighing the fresh masses, the materials were packed in paper bags and placed in a forced air circulation oven at 60ºC ± 5 until constant mass. The dry masses were weighed, and the mass allocations in leaves, stems, and roots were calculated. By using data on the mass and leaf areas, the ratio of the leaf, specific leaf area, specific leaf mass, and shoot to root ratio were calculated according to Benincasa [17].

Survival data were transformed into $\sqrt{(x + 0.5)}$ for normalization, and if significant, the actual values were presented. The data were submitted to analysis of variance and, if significant by the F test, the averages were submitted to Student's t test for luminosity and the test of Tukey for cuttings types at 5% of probability, all procedures using the statistical program SISVAR® [18]. Subsequently, a complementary multivariate analysis of principal components was performed using variance and covariance matrices, using PAST software 3.21 [19].

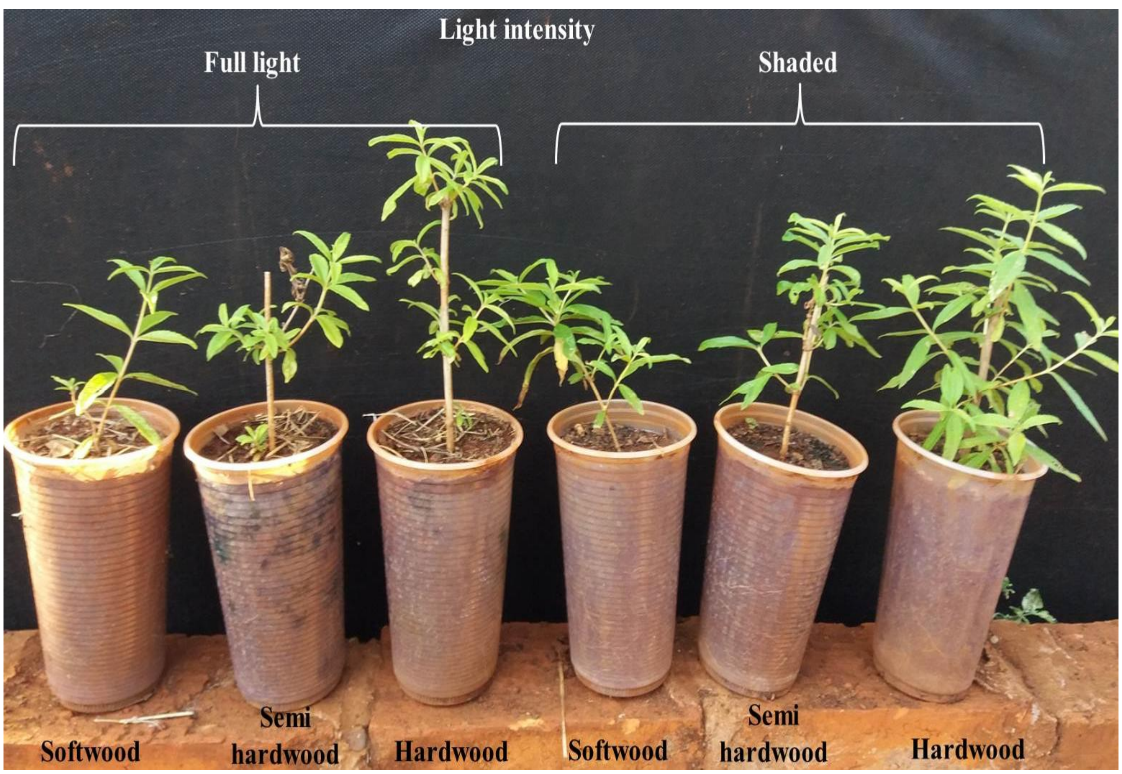

Fig. 1. Cidró cuttings type and light intensity, 60 days after cutting

3. RESULTS AND DISCUSSION

The highest survival percentages (86 and 82%) of cidró cuttings occurred when hardwood cuttings were used, regardless of the light intensity. On the other hand, the lowest survivals were those with softwood cuttings, both at full light and shaded conditions (15.36 and 41.08%, respectively) (Fig. 2).

The highest survival of the hardwood cuttings is attributable to their greater diameter, which guarantees greater accumulation of reserves to be used in the formation of new organs and the homeostatic capacity of the establishment. Pacheco and Franco [11] report that the influence of diameter on plant survival can be explained by differences in carbohydrate content and lignification of cuttings. Santos et al. [9] evidenced that medium and basal cuttings of *Lippia gracilis* Schauer presented higher survival and growth, similar to those obtained in this study.

The fresh and dry masses of leaves, stems and roots were influenced only by the type of cutting (Table 1), being higher in the cuttings from hardwood, with increases in fresh and dry mass by 0.4037 and 0.1812 (leaves); 0.0787 and 0.0538 (stem) and 0.1525 and 0.0788 g plant^{-1} (root), respectively, in relation to the softwood cuttings, which were those that had the lowest yields.

The most significant developments may be associated with larger diameters of the cuttings. This is because hardwood species have a greater accumulation of reserves, which can be translocated and used in root formation [20], as observed in this study. Similar results were observed by Santos et al. [9] in which semi-hardwood and hardwood cuttings of *Lippia gracilis* Schauer presented greater vegetative development than the apical ones. Another essential fact to be emphasized in the responses of cuttings production is the energy expenditure to maintain the leaves in apical cuttings, when compared to the others, that is, this causes a larger increase in the formation and accumulation of the mass of the aerial part and roots [9].

As for the mass allocation in different organs of cidró cuttings, it was observed that the softwood cuttings presented higher root allocation in the two light environments (Fig. 3a and Fig. 3b). It is due to the greater growth and tuberosity of the main root, providing an efficient absorption and less loss of water by transpiration in the leaves [21] and that under full light conditions, a greater increase in leaves and smaller stem of this same type of cuttings were observed. Yet, in a shaded environment, it was observed the standardization of the mass partition and allocation, indicating that the cidró presents physiological plasticity to the crop conditions.

The leaf and root areas were influenced only by the types of cuttings, and the highest values occurred using hardwood cuttings, with an increase of 26.76 and 6.48 cm^2 plant^{-1}, in relation to the softwood plants (Table 2). The number of shoots was influenced only by the light intensity, reaching 0.66 buds plant^{-1} in full light. Yet, the number of leaves was influenced by light factors alone, with the highest values in hardwood and semi-hardwood cuttings under full light. On the other hand, the length of the shoots was not influenced by the factors under study, with a mean of 3.32 cm.

The largest numbers of shoots and leaves in the cuttings under full light condition (Table 2) are caused by the greater incident solar radiation. This is because plants exposed to full light conditions tend to exhibit greater assimilation of CO_2 [22], consequently, greater production of photoassimilates and increase of new organs, such as leaves and shoots. Thus, the growth, number of shoots and the adaptation of the plant to the environment are related to its photosynthetic efficiency [23].

The largest leaf and root areas and number of leaves from the hardwood and semi-hardwood cuttings are due to the amount of reserves as a function of the larger diameters, similar to the masses. Leaf characteristics are essential in the analysis of plant growth since they are associated with the greater area of interception of solar radiation, aiming to optimize higher photosynthetic capacity [24].

The highest root length (RL) occurred from the softwood cuttings in full light conditions, reaching 11.46 cm, when compared to the smallest root length, from hardwood cuttings in a shaded environment (Table 3) because these cuttings displayed a greater amount of leaves when compared to the others. Hence, a greater interception of light occurs from the beginning of the burial of the cuttings, therefore, assuring higher production and partition of photoassimilates for the other organs. Also, endogenous auxin is more concentrated in the apical meristems of plants [25,26], which ensures faster distribution to the base of the cutting, forming adventitious roots. In addition, the higher RL can be explained by the fact that, under high irradiance, water loss of the substrate occurs due to the higher temperature, which causes the plants to present strategies for exploration of water and nutrients in order to perform the metabolic processes.

The physiological indices of leaf area ratio (LAR) and specific leaf mass (SLM) were influenced only by light intensity, with higher values in full light sites (Table 4). As regards LAR, an increase of 46.11 cm^2 g^{-1} occurred in relation to the full light treatment, and for SLM reaching 0.0037 g cm^{-2}. The leaves of plants exposed to high irradiance tend to be smaller and thicker with compact mesophylls and reduced intercellular spaces, being related to the regulation of light radiation and CO_2 diffusion. Therefore, it provided better photosynthetic efficiency [27]. The specific leaf area was influenced only by the types of cuttings, in which an increase of 31.84 cm^2 g^{-1} was observed in semi-hardwood cutting when compared with the softwood cuttings. In this context, the specific leaf area is a great parameter for the plasticity of plant, due to the relationship with increased photosynthetic rate [28], evidencing the answer found. On the other hand, the APRR was solely influenced by the luminosity and the types of cuttings with higher values under full light treatment and hardwood cuttings, surpassing by 0.70 those of lower value, that is, of the softwood portion. However, the biomass distribution between root and area part, net carbon assimilation and leaf area ratio, are indicative of the morphophysiological mechanisms of species exposed to light changes [29].

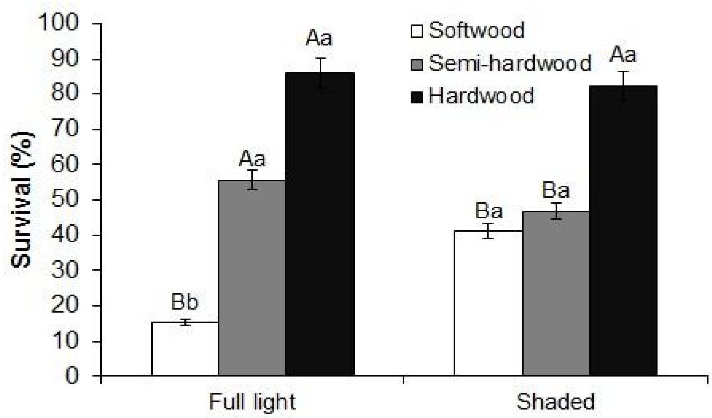

Fig. 2. Survival of cidró cuttings propagated by different types of cuttings under light intensity at 60 days after cutting

Bars with the same letters do not differ from each other in the Student t-test for light intensity and Tukey test for stakes (p> 0.05)

Table 1. Fresh (FM) and dry (DM) mass of leaves, stems, and roots of cidró according to the types of cuttings and light intensity at 60 days

Light intensity	Leaves		Stems		Roots	
	(g plant^{-1})					
	FM	DM	FM	DM	FM	DM
Shaded	0.40 a	0.19 a	0.08 a	0.04 a	0.12 a	0.08 a
Full light	0.39 a	0.22 a	0.07 a	0.05 a	0.20 a	0.12 a
Types of cuttings						
Softwood	0.20 b	0.12 b	0.03 b	0.02 b	0.09 b	0.06 b
Semi-hardwood	0.39 b	0.19 ab	0.09 ab	0.05 ab	0.16 ab	0.11 ab
Hardwood	0.61 a	0.30 a	0.11 a	0.07 a	0.24 a	0.14 a
C.V. (%)	39.64	41.59	68.61	65.53	53.99	43.31

Means followed by the same letter in the columns are not different from each other by the Student t-test and for light intensity by the test of Tukey for the cuttings (p>0.05)

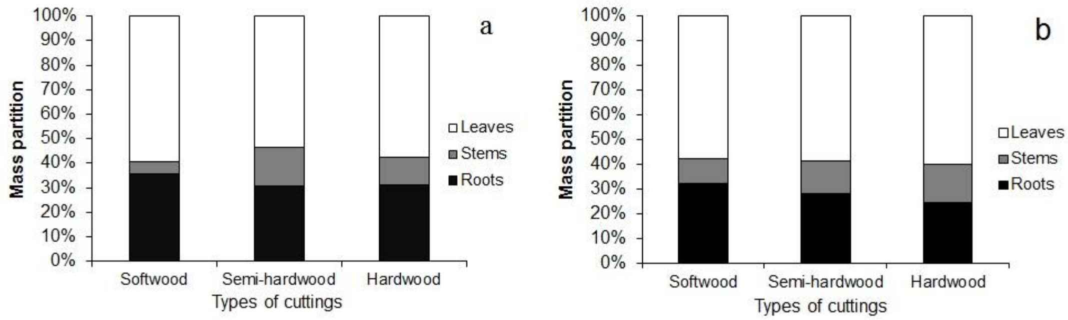

Fig. 3. Partitioning of dry mass of cidró, according to the types of cuttings and light intensity, A – Full light; B – Shaded, 60 days after cuttings

Table 2. Leaf area (LA), root area (AR), number of shoots (NS), number of leaves (NL) and bud length (BL) of cidró according to the types of cuttings and light intensity at 60 days

Light intensity	LA	AR	NS	NL	BL
	(cm^2 plant^{-1})		(unit. plant^{-1})		(cm)
Shaded	36.43 a	9.89 a	1.19 b	12.25 b	3.79 a
Full light	26.64 a	12.61 a	1.85 a	17.68 a	2.86 a
Types of cuttings					
Softwood	17.81 b	7.30 b	1.21 a	11.97 b	2.10 a
Semi-hardwood	32.24 ab	12.68 a	1.58 a	16.22 a	3.49 a
Hardwood	44.57 a	13.78 a	1.76 a	16.70 a	4.38 a
C.V. (%)	41.60	45.31	35.92	19.61	61.84

Means followed by the same letter in the columns are not different from each other by the Student t-test for light intensity and by the test of Tukey for the cuttings (p>0.05)

Table 3. Root length of cidró according to the types of cuttings and light intensity at 60 days

Types of cuttings	Root length (cm)	
	Shaded	Full light
Softwood	6.09 Ab	16.54 Aa
Semi-hardwood	5.32 Aa	8.65 Ba
Hardwood	5.08 Aa	5.95 Ba
C.V. (%)	45.50	

Means followed by the same uppercase letter in the columns and by lowercase letter in the rows are not different from each other by the Student t-test and for light intensity and by the test of Tukey for the cuttings (p>0.05)

Table 4. Leaf area ratio (LAR), specific leaf area (SLA), specific leaf mass (SLM), shoot to root ratio (APRR) of cidró according to the light intensity and types of cuttings at 60 days

Luminosity	LAR	SLA	SLM	APRR
	(cm^2 g^{-1})		(g cm^{-2})	
Shaded	114.76 a	195.06 a	0.0063 b	2.14 b
Full light	68.65 b	121.52 a	0.0100 a	2.61 a
Types of cuttings				
Softwood	84.71 a	144.49 b	0.0083 a	1.98 b
Semi-hardwood	99.39 a	176.33 a	0.0075 a	2.48 ab
Hardwood	91.01 a	154.05 b	0.0086 a	2.68 a
C.V. (%)	12.80	10.29	11.57	17.59

Means followed by the same letter in the columns are not different from each other by the Student t-test for light intensity and by the test of Tukey for the cuttings (p>0.05)

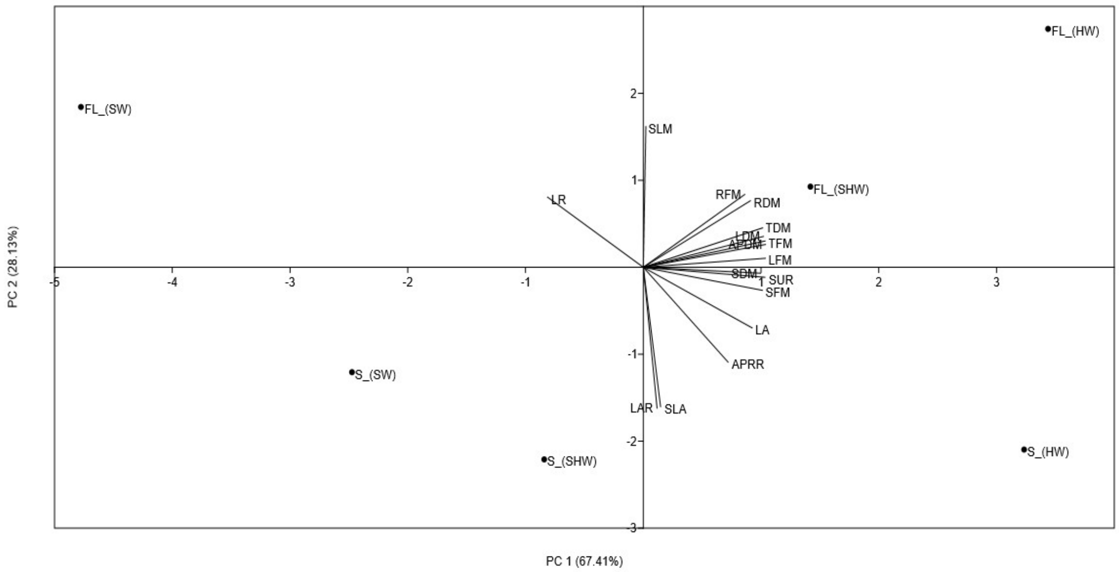

Fig. 4. Principal components analysis of vegetative characteristics in cidró propagated by stem cuttings (SW = Softwood, SHW = Semi-hardwood and HW = Hardwood), under light intensity (FL = Full light and S = shaded)

Principal components analysis indicated 95.54% of the remaining variability of the data, with factorial scores above 0.20 (Fig. 4), where 67.41% and 28.13% are represented by the principal components 1 and 2, respectively. By considering the factorial charges of the eigenvectors in descending order, the characteristics of higher constitutional weights were APDM, LFM, TFM, and SUR, for PC1, and LAR, SLM, SLA and APRR for PC2. In this sense, under these experimental conditions, it was verified that the most representative characteristics in a qualitative way were the mass and the physiological indices of the cuttings.

4. CONCLUSION

The vegetative propagation of cidró can be carried out using the hardwood portion of the cuttings branch, under full light. These results provide information for the production of cidró on a large scale, aiming at its potential medicinal use.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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