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2.4-Dichlorophenoxyacetic acid and sucrose in rhizogenesis maintenance and induction in cuttings of cagaita and mangabeira from cerrado

The Cerrado has a vast diversity of fruit plant species. Among them, the cagaita and mangabeira trees stand out because of their fruits nutritional potential. They propagate through seeds, a method that generates genetically distinct plants. Thus, it would be of interest to propagate these plants through vegetative propagation techniques aiming to select superior and identical genotypes from the mother plant. Therefore, the objective was to establish a methodology for maintenance, survival, and rooting induction of Eugenia dysenterica and Hancornia speciosa var. pubescens in nutrient solution with different sucrose concentrations added with 2.4-dichlorophenoxyacetic auxin (2.4-D). In the cagaita and mangabeira tree cuttings, five sucrose concentrations were evaluated: 0, 5, 10, 15, and 20% for the first; and 0, 10, 20, 30, and 40 % for the second species. In addition to these, the control was deionized water. To the sucrose solution was added 190 g L-1 NH4NO3, Ca3ClO, K2SO 4, and 3.00 mg L -1 of 2.4-D. We also performed a second test for cagaita tree, which was evaluated different doses of 2.4-D: 0, 0.5, 1.0, 1.5, and 2.0 mg L-1; and control (deionized water). In all experiments, the completely randomized design was used. We collected data on survival, contamination, cuttings with permanent leaves, base oxidation, callus formation, and root starts. In the first cagaita tree experiment, cuttings survival was higher than 80%. In the second trial, the use of 2.4-D did not favor the survival of the cuttings, nor did it promote the formation of root starts. For mangabeira was observed survival values of 100% in all treatments evaluated, and root starts were also found in its herbaceous cuttings.

Keywords: Fruit tree; Propagation; Sucrose; Auxins.

Ácido 2.4-dichlorophenoxyacetico e sacarose na manutenção e indução de rizogênese em estacas de cagaita e mangabeira do cerrado

O Cerrado apresenta enorme diversidade de espécies vegetais frutíferas. Dentre elas estão a cagaita e mangabeira que se destacam pelo potencial alimentício de seus frutos. Estas são propagadas por meio de sementes. Este método gera plantas geneticamente distintas. Assim, seria interessante a propagação destas plantas por meio de técnicas de propagação vegetativa visando selecionar genótipos superiores e idênticos à planta matriz. Portanto, objetivou-se estabelecer uma metodologia para a manutenção, sobrevivência e indução ao enraizamento de estacas semilenhosas de Eugenia dysenterica e herbáceas de Hancornia speciosa var. pubescens em solução nutritiva com diferentes concentrações de sacarose adicionados da auxina 2,4-diclorofenoxiacético (2,4-D). Nas estacas de cagaita e mangabeira avaliaram-se cinco concentrações de sacarose: 0; 5; 10; 15 e 20% para a primeira e 0; 10; 20; 30 e 40% para a segunda espécie. Além destes foi avaliada a testemunha (água deionizada). À solução de sacarose foram acrescidos NH4NO3 a 190 g L-1; Ca3ClO; K2SO4 e 3,00 mg L-1 de 2,4-D. Além destes ensaios foi realizado um segundo para a cagaita em que se avaliou doses de 2,4-D: 0; 0,5; 1,0; 1,5; e 2,0 mg L-1; e testemunha (água deionizada). Em todos os experimentos foi adotado delineamento inteiramente casualizado. Ao fim dos ensaios foram coletados dados de: sobrevivência; contaminação; estacas com folhas permanentes; oxidação da base; formação de calo e primórdios radiciais. No primeiro experimento de cagaita a sobrevivência das estacas foi superior a 80%. Já no segundo ensaio o uso de 2,4-D não favoreceu a sobrevivência das estacas de cagaita, bem como não promoveu a formação de primórdios radiciais. Para a mangabeira observou valores de sobrevivência de 100% em todos os tratamentos avaliados. Também foi constatada presença de primórdios radiciais nas suas estacas herbáceas.

Palavras-chave: Frutífera; Propagação; Sacarose; Auxinas.

Topic: **Uso Sustentável da Biodiversidade**

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INTRODUCTION

The Cerrado is a complex biome and place of several native fruit trees. These species are essential in the local diet as a food supplement in rural areas as they are sources of protein, fiber, vitamins, calcium, iron, phosphorus, and fatty acids (ALMEIDA et al., 1998). More than ten types of edible fruits are regularly consumed by the local population and sold in urban centers such as pequi (*Caryocar brasiliense*), buriti (*Mauritia flexuosa*), Mangaba (*Hancornia speciosa*), cagaita (*Eugenia dysenterica*), bacupari (*Salacia crassifolia*), cajuzinho do cerrado (*Anacardium othonianum*), araticum (*Annona crassiflora*) and seeds of barú (*Dipteryx alata*) (MMA, 2019).

Cagaita (*Eugenia dysenterica*) is a native species from Cerrado. Although it has applicability for the extraction of essential oils (CAMILO et al., 2016), its primary use is related to the nutritional potential of its fruits. According to Chaves et al. (2006a), it can be consumed fresh or used as ingredients for juices, jams, ice cream, and popsicles.

Mangabeira is a tropical plant native to Brazil and found in various regions of the country. This fruit has aromatic, tasty, and nutritious fruits, with extensive market acceptance, both for fresh consumption and industry (SOARES et al., 2007). Latex can be extracted from the tree, which may vary in production depending on the time of the year (ARRUDA et al., 2016). In the state of Goiás, there are two varieties of *Hancornia speciosa*, var. *gardneri* and *pubescens*, which differ by the *gardneri* having glabrous leaves and *pubescens* having pubescent leaves, on the abaxial face (CHAVES et al., 2006b).

Seedling production of these two species by seeds is vital for germplasm bank formation and selection of superior genotypes (VIEIRA et al., 2015). However, it is in the vegetative propagation that the production of superior clones can be performed and thus reducing the time of fruit production. Stem cuttings are a method of vegetative propagation. It has been mainly used for the advantages of maintenance of genotypic characteristics and seedling production in species that present difficulties in sexual propagation, which may be caused by seed trauma due to rotating fruiting and dormancy (OLIVEIRA et al., 2001).

Treating stakes with synthetic auxin may favor the successful propagation in cuttings of some species, especially those that have difficulty to root. Vegetative propagation is a way of obtaining early, healthy, and uniform seedlings. This process is most suitable when it comes to producing fruit with quality and speed (FACHINELLO et al., 2005). Sucrose is an essential carbohydrate in plant physiology. It may be responsible for retarding the degradation of proteins, lipids, and ribonucleic acids (NOWAK et al., 1991).

According to Oliveira et al. (2015), among the synthetic auxins used, there is indole-3-butyric acid (IBA), 1-naphthalenacetic acid (ANA), 2.4-dichlorophenoxyacetic acid (2.4-D), and 2-naphthoxyacetic acid (NOA). These are widely used to induce rooting in vegetative propagules.

Studies to assess the use of auxins in woody and herbaceous cuttings have been performed (MACHADO et al., 2010; SASSO et al., 2010; REGINA et al., 2012; VIGNOLO et al., 2014; OLIVEIRA et al., 2015; GALVÃO et al., 2016; PAIXÃO et al., 2016; RINALDI et al., 2017; JESUS et al., 2018; SOUZA et al., 2018; VIEIRA et al., 2018; TELEGINSKI et al., 2018). However, propagation protocols of cagaita and mangabeira cuttings are not yet established; therefore, research is needed to define a useful methodology to include these species in the ranking of cultivated plants, as well as for their conservation and maintenance in germplasm bank and clonal gardens.

In this sense, this study aimed to establish a methodology for the maintenance, survival and rooting induction of herbaceous cuttings of *Hancornia speciosa* var pubescens and *Eugenia dysenterica* semi-wood cuttings in nutrient solution with different concentrations of sucrose added with synthetic 2,4-dichlorophenoxyacetic auxin.

MATERIALS AND METHODS

Cuttings of *E. dysenterica* from Cerrado

We collected cuttings from productive plants, located in an experimental area of the Federal University of Goiás, installed in 1988 through seeds propagation. The collection of semi-hardwood cuttings was carried out in the morning and placed in buckets of water to avoid possible damage related to dehydration. They were 20 cm long, 3 to 4 mm in diameter, and with two pairs of leaves, cut in half. At the bottom, a diagonally cut was made. The first trial was installed in September of 2018, which evaluated five different sucrose concentrations: 0, 5, 10, 15, and 20%, and the control (deionized water). There were five repetitions and five cuttings per plot. To the sucrose solution was added 190 g L^{-1} NH_4NO_3, Ca_3ClO, K_2SO_4, and 3.00 mg L^{-1} of 2.4-D.

We also installed the second trial in September, and the following doses of 2.4-D were evaluated: 0, 0.5, 1.0, 1.5, and 2.0 mg L^{-1}; and a control (deionized water). The experiments were conducted in a completely randomized design. The cuttings were placed inside plastic cups containing 50 mL of the solution and maintained in a controlled ambient with the temperature at 25 °C. During the studies, water was sprayed on the cuttings. In the second trial, a plastic bag was placed around the cuttings to create a microclimate similar to that of a greenhouse, thus reducing the perspiration by the leaves favoring the green color, leaf retention, and cuttings survival.

At the end of the first trial (seven days) and second trial (four days), the following variables survival, contamination, number of permanent, green and hydrated cuttings leaf, base oxidation, callus formation, and root starts. Descriptive data analysis was performed with the average values for the analyzed variables.

Cuttings of *H. speciosa* from Cerrado

Mangabeira cuttings of *pubescens* variety were collected in the spring, approximately 10 cm long and taken to the Phytotechnic Laboratory of UFG – Goiânia, Brazil, for asepsis procedures. The cuttings were diagonally cut at the bottom and also at the stem apex, and only two pairs of leaves from the apex down were kept. Leaves had 2/3 cut off. Solutions of 0, 10, 20, 30 and 40% of sucrose diluted in deionized water were prepared. To this solution was added 190 g L^{-1} NH_4NO_3, Ca3ClO, K_2SO_4, and 3.00 mg L^{-1} of 2.4-D, and the cuttings put into this solution.

Each treatment consisted of four repetitions. After immersed in the solution, the cuttings were placed in a greenhouse at an average temperature of 28 ºC and below a 50% shaded mesh. Survival, contamination, number of permanent and/or senescent leaves pair (SLP), base oxidation and green base, callus formation, and root starts, variables were evaluated at eight days after the installation of the test.

After rooting induction, the mangaba cuttings were transferred to a 50 cm^3 tubes filled with Bioplant substrate, then placed in a greenhouse with intermittent mist irrigation of 15-second cycle and 3-minute intervals for 12 hours. Weekly evaluations for rooting index, survival, budding, and persistence of leaves were performed until the 30th day after planting. We submitted the data to descriptive statistics with a presentation of mean indices.

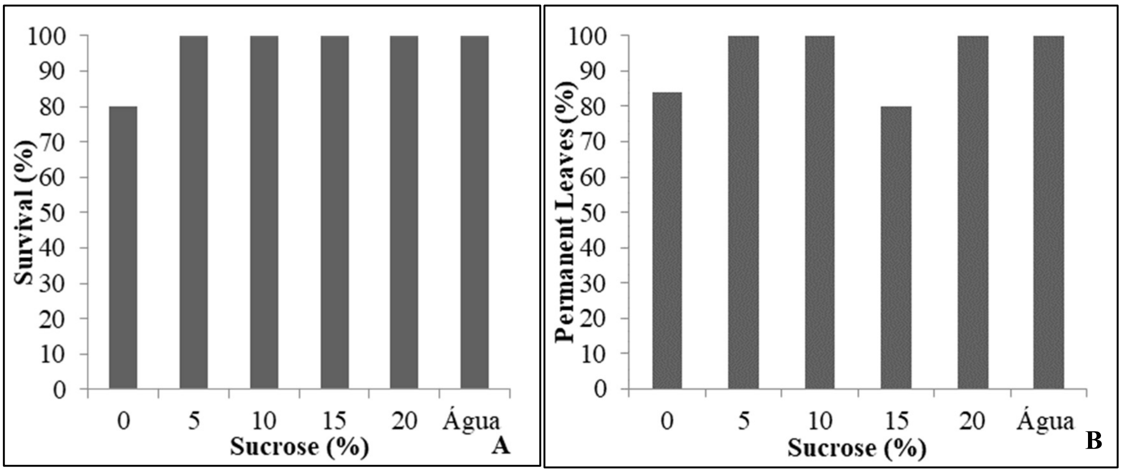
RESULTS

Eugenia dysenterica

No callus nor root starts were found in any cutting of the six treatments evaluated at the end of the first trial (seven days). The solutions contained in the cups had no contamination, and the bases of the dead cuttings were dark in color, similar to oxidation.

The different sucrose concentrations analyzed showed survival values higher than 80% (Figure 1-A). The percentage of cuttings with permanent leaves were also above 80% (Figure 1-B, C). Besides a higher percentage of permanent leaves (>80%), some were not hydrated, the treatment without sucrose presented the lowest value. The use of sucrose helps to maintain hydrated leaves and, consequently, the maintenance of viable propagules for a longer time (Figure 2).

However, the results showed that the use of deionized water was a viable method to preserve cuttings since it had 100% survival and permanent leaves, 88% of green leaves, and 76% of hydrated leaves (Figure 1-D). Thus, there is no need to add sucrose to the solution.



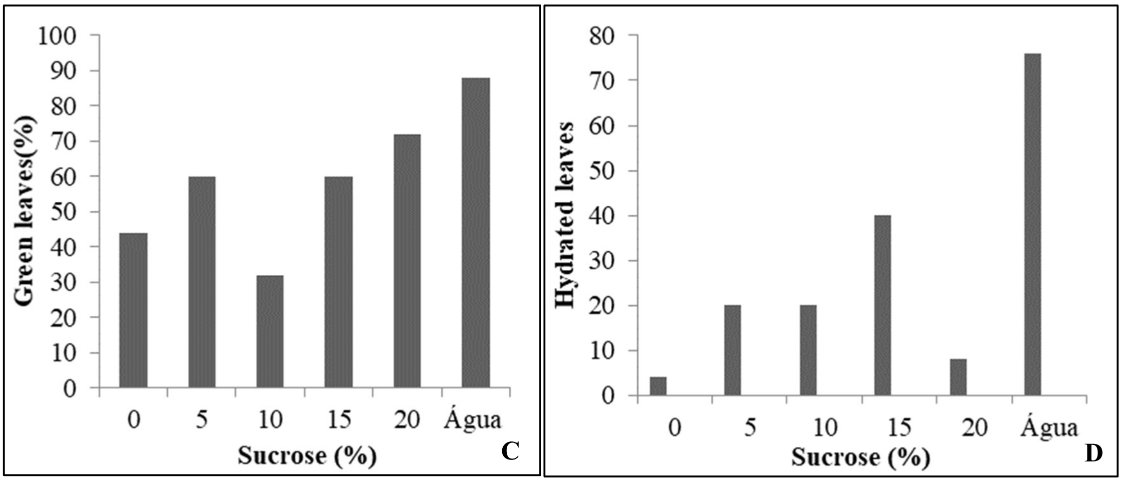


Figure 1: Mean values in % for the variables: Survival (A), Permanent Leaves (B), Green Leaves (C), and Hydrated Leaves (D) from *Eugenia dysenterica* cuttings.

Figure 2: *Eugenia dysenterica* cuttings deposited in plastic cups.

There was no significant effect on the different treatments evaluated in the second trial. Treatment 6 (Table 1) used only deionized water and maintained 100% survival of cuttings, as they presented two pairs of leaves without symptoms of dehydration and contamination. This fact may have been due to the non-adaptation of cagaita cuttings by substrates that are constituted by nutrient and root solutions or the time of collection was not the most appropriate for the production of cuttings under these conditions.

The use of 2.4-D did not favor the maintenance of viable propagules and low survival rates were observed in all treatments using this substance (Table 1). It was probably due to high doses of the phyto-regulator, which performed the reverse process, i.e., instead of inducing rooting or callus, 2.4-D played a role of herbicide, leading most of the cuttings to death.

Table 1: Survival and Permanent Leaves (PL) in cagaita cuttings.

Treatments	Variables	
Sucrose + 2.4-D (mg L^{-1})	Survival (%)	PL (%)
1 (0.0)	0.00	0.00
2 (5.0)	20.00	20.00
3 (10.0)	4.00	4.00
4 (15.0)	20.00	20.00
5 (20.0)	12.00	8.00
6 Control (H_2O)	100.00	100.00

Hancornia speciose

No contamination (Table 2) was found in the cuttings of Treatment 1 (Figure 3), unlike the others, it may be due to the lack of sucrose added to the solution. Significant survival rates were observed in all treatments evaluated with both sucrose and the absence of it.

The only treatment with cuttings where no green bases were not observed was 2, in which the dose of sucrose concentration was 5%. Treatment 1 presented 80%, 75% for Treatment 3, 75% in Treatment 4, and 10% for Treatment 5. For treatments 3, 4 and 5, all showed an absence of oxidized base and in treatment two, all of the cuttings had this phenomenon.

Table 2: Average Data for Survival Rate (Su.); Contamination (Cont.); Leaf permanence (Perm); Leaf senescence (Senesc); oxidized or green base; and root starts (RS) in pretreatment of *H. speciosa* var. *pubescens* at different sucrose concentrations in nutrient solution plus 2.4-D.

			NLP		NLP2			Foot	
Values %									
Sucrose (%)+2.4-D(mg L^{-1})	Su.	Cont.	Perm.	Senesc	Perm. (tip)	Senesc.	Oxidation	Green	RS
1 (0.0)	100	0	10	10	60	0	10	80	0
2 (10.0)	100	75	75	10	100	10	100	0	10
3 (20.0)	100	10	10	0	100	100	0	75	50
4 (30.0)	100	75	10	50	50	10	0	75	0
5 (40.0)	100	75	10	10	100	75	0	10	75

Figure 3: Mangabeira cuttings in evaluation.

The roots starts were present in treatments 2, 3, and 5 (Table 2, Figure 4). The sucrose concentrations were 10, 20, and 40%, respectively (Figure 5). The use of sucrose in solutions to aid seedling maintenance and rooting induction can be an exciting alternative when working with cuttings.

In this sense, Machado et al. (2010) working with cuttings obtained from in vitro shoots grown in QL-culture medium supplemented with 15, 30, and 45 g L^{-1} of sucrose reported rooting in cuttings at all tested sucrose concentrations. Furthermore, they observed a high survival rate (100%) during the ex-vitro acclimatization that was obtained when the shoots were cultivated in the culture medium supplemented with 45g L^{-1} sucrose.

Figure 4: Roots starts in Mangabeira cuttings var. *pubescens* after eight days in nutrient solution plus sucrose and 2,4-D.

Figure 5: Herbaceous cuttings of *H. speciose* var. *pubescens* after eight days in nutrient solution plus sucrose and 2.4-D.

DISCUSSION

Eugenia dysenterica

The maintenance of leaves is important because they play a fundamental role in the survival and rooting of cuttings. Also, found that leaves are essential for adventitious root formation. According to Vignolo et al. (2014), it is likely that rooting and survival of leaf cuttings are related to the synthesis of phenolic compounds by shoots, and studies indicate certain phenolic compounds, such as caffeic acid, catechol and chlorogenic, interact with auxins inducing root starts.

The use of phyto-regulators does not always assist in the vegetative propagation of plants. In studies with gabiroba (*Campomanesia xanthocarpa*) Teleginski et al. (2018) found that the technique of *alporquia* using the plant regulator is indole-3-butyric acid (IBA) was not efficient because it did not promote rooting. For the production of seedlings of *Vittis* spp. Regina et al. (2012) found that the use of IBA for rootstock rooting did not positively influence seedling production.

The cuttings kept both their first and second leaves permanent. The presence of leaves helps to observe their behavior throughout the study, since the absence of abscission may indicate that the cutting still has the potential to form roots. Pacheco et al. (2008) argue that leaf fall becomes a negative point for cuttings propagation, as leaf retention can reduce cuttings death as they are natural sources of carbohydrates and auxin.

Yamamoto et al. (2010) observed that 2,000 mg L^{-1} of IBA provided the best rooting characteristics in herbaceous cuttings of *Psidium guajava* L. '21st Century' and the use of talcum powder was more efficient than alcohol. IBA is an auxin with promising responses in many fruit species. In this study, when using auxin 2.4-D at low dosages, it was found a positive response to rooting induction.

Evaluating the use of grafting and root induction by bottom heating to produce grapevine seedlings, Jesus et al. (2018) found that by heating the bottom of the cuttings, it induced a high rate of rooting (80%) but did not interfere with the percentage of seedling formation. The pretreatment with IBA (2,000 mg L^{-1} IBA in 50% hydroalcoholic solution) was unfavorable to the seedling formation.

Hancornia speciosa

In this sense, Machado et al. (2010) working with cuttings obtained from in vitro shoots grown in QL-culture medium supplemented with 15, 30, and 45 g L^{-1} of sucrose reported rooting in cuttings at all tested sucrose concentrations. Furthermore, they observed a high survival rate (100%) during the ex-vitro acclimatization that was obtained when the shoots were cultivated in the culture medium supplemented with 45g L^{-1} sucrose.

In besides to sucrose, the use of 2.4-dichlorophenoxyacetic acid (2.4-D) helped in the process of root formation. The promising use of this auxin can be observed when evaluating the present study in its unfolding. Other authors such as Galvão et al. (2016) evaluating the action of auxin IBA in Pitaia (*Cereus undatus* Haworth) cuttings found significant differences in phytotechnical characteristics as number and length of cladodes, fresh root biomass and shoot. They observed that the range between 1,776.33 and 2,299 mg L^{-1} of IBA provided more significant growth of Pitaia seedlings.

According to Paixão et al. (2016) when evaluating the potential of policaulia in avocado seeds according to seed mass and adventitious rooting of polycultural shoots with and without the use of auxin type IBA found that stems above 20 cm not treated with IBA resulted in better quality avocado seedlings. Thus, the use of IBA (2,000 mg L^{-1}) does not affect the rooting and growth of avocado tree seedlings. Analyzing cuttings propagation of Dovyalis using 1,666 mg L-1 of AIB, Rinaldi et al. (2017) realized that this plant regulator favored the rooting of the cuttings of this fruit tree. Cuttings of 15 and 20 cm with four leaves benefit the vegetative propagation of *Dovyalis*.

In a study with Jabuticabeira (*Plinia cauliflora*) cuttings, Sasso et al. (2010) tested the rooting efficiency of woody cuttings using four concentrations of IBA (0; 2,000; 4,000 and 6,000 mg L^{-1}). The authors observed that rooting of woody cuttings is dependent on the application of IBA, and the highest rooting percentage (50%) was obtained with the highest concentration of IBA (6,000 mg L^{-1}) combined with the vertical cut. For herbaceous apical cuttings, rooting was no higher than 10%.

Over the first week of evaluation in the greenhouse, the cuttings had a survival rate of 100%, with leaf maintenance. In this period, no sprouts were observed. In the second week, there was a gradual fall of the leaves and senescence of cuttings from the base proximal to the substrate. At 30 days, 60% of the cuttings had lost their leaves and necrosis from the base to the middle third. No root development indexes were observed. Pena et al. (2015), in studies with small cuttings from small shoots, originated from adult *E. uniflora* grafting, found 96.4% loss in cuttings harvested in spring.

The survival of Mangabeira cuttings for a short period may be a consequence of environmental and genetic factors. However, because this is an undomesticated species, matrix plant conditions, or as in the present study, the type and amount of endogenous hormones and the growth regulators in which the cuttings were treated, may have contributed to cuttings did not continue to root development when planted on substrates.

According to Raven (1996), the growth and development of an organism are not possible without efficient communication between cells, tissues and organs, and the regulation and coordination of metabolism, growth and morphogenesis depend on the action of chemicals, called hormones. Therefore, all factors related to vegetative propagation must be studied to make them competitive in genetic improvement and in agronomic management.

CONCLUSIONS

Under the conditions which the study was conducted, we conclude that: Cagaita: There is no need to use sucrose solutions with added NH_4NO_3, Ca3ClO, K_2SO_4, and 2,4-D for rooting maintenance and induction in semi-hardwood cuttings. The use of 2.4-D in the treatment of cuttings should be used at lower doses than this study since there was a large number of dead cuttings. We recommend that the treatment of cuttings with phytoregulators or with other substances be performed in a shorter period than in this study.

The period of one week (1st experiment) and four days (2nd experiment) negatively influenced the maintenance of the propagules, since there was a large number of cuttings with dehydrated leaves. Research aiming to study the effect of substances on callus induction and root primordia in semi-woody cuttings should be conducted in an intermittent fogging environment, aiming at a higher percentage of cuttings survival.

Mangabeira: Studies were promising for T2, followed by T5 and T3, respectively, for induction of root starts. For propagule survival, all treatments maintained 100% survival for the period evaluated. More studies are needed to promote the establishment of methodology for vegetative propagation of *H. speciosa* var. *pubescens* from Cerrado, Brazil. For cuttings production of both Cagaita and Mangabeira, studies that use cuttings from different origins and collected at different times, are essential. In addition to these factors, find different substrates and plant regulators in order to define a methodology that is efficient in producing seedlings of these endemic species and can be used by the farmers.

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