

## Advances and perspectives in the propagation of mangabeira (*Hancornia speciosa* Gomes) – review on a tropical fruit tree with socioeconomic importance

Augusto Vinicius de Souza Nascimento<sup>1\*</sup>, Paulo Augusto Almeida Santos<sup>1</sup>, Ana Veruska Cruz da Silva Muniz<sup>2</sup>, Ana da Silva Lédo<sup>2</sup>

<sup>1</sup>Federal University of Sergipe, Brasil  
<sup>2</sup>Embrapa Tabuleiros Costeiros, Brasil

SILVICULTURE

### ABSTRACT

**Background:** *Hancornia speciosa* is a species known for its medicinal and nutritional properties. Despite being a plant native to several regions of Brazil, some aspects of its production system are not yet well defined. Large-scale asexual propagation stands out, making it difficult to establish commercial orchards with materials with superior characteristics. In this context, this article aimed to investigate the scientific production related to the propagation and conservation of the mangabeira, using a mixed methodology of bibliometric analysis and literature review. The Scopus and Web of Science databases were consulted and the VOSviewer software was used to map the cooperation network between authors, institutions, and keywords.

**Results:** The study included 72 articles and found a significant increase in scientific production on mangabeira propagation and conservation since 2009. Most studies focused on seed technology, seedling production, *in vitro* propagation and conservation, and cryopreservation. Additionally, it was observed that mangabeira seeds do not tolerate a reduction in moisture content, and the combination of materials for the composition of the substrate, such as coconut fiber, manure, and sand, can provide an adequate basis for developing seedlings. It was also observed that MS culture medium, complete or with half saline concentration, or WPM medium can be used for *in vitro* germination and initial seedling growth.

**Conclusion:** Through this review, we concluded that the mangabeira presents substantial potential for the development of innovative propagation techniques, with a special focus on methods that facilitate the clonal propagation of the species, such as grafting, rooting cuttings, and micropropagation. It is expected that future research will expand knowledge about the *in vitro* and *ex vitro* propagation of this species, contributing to the expansion of commercial plantations with a focus on adding to the processing of its fruits.

**Keywords:** Apocynaceae; germination; mangaba; micropropagation; native fruit.

### HIGHLIGHTS

After 2009 there was an increase in studies on mangabeira propagation/conservation. A total of 72 scientific articles were identified and included in the review. The studies involving the vegetative propagation of mangabeira in broad are still scarce. Cloning by cuttings on a large scale may be a future trend.

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\*Corresponding author: augustovinicius11@gmail.com

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## INTRODUCTION

The mangabeira tree (*Hancornia speciosa* Gomes) is a fruit-bearing and medicinal tree that is native to Brazil and belongs to the Apocynaceae family. It has a wide distribution throughout the country, occurring in the Caatinga, Amazon, Cerrado, and Atlantic Forest biomes, and it is also found in Bolivia, Peru, and Paraguay (Coradin *et al.*, 2018). This species holds great economic importance as its fruit, mangaba, is consumed fresh or processed by small agro-industries to make juices, popsicles, ice creams, jams, and other products. It serves as an important source of income for traditional communities, such as mangabeira gatherers (Soares *et al.*, 2011, Chaves- Almeida *et al.*, 2022, Nunes *et al.*, 2022a). Furthermore, the mangabeira tree exhibits significant pharmacological potential due to the presence of compounds like rutin and chlorogenic acid, which can be found in its leaves, barks, fruits, and latex, possessing anti-inflammatory and antioxidant properties (Leite *et al.*, 2020; Nassiri-Asl *et al.*, 2017; Torres-Rêgo *et al.*, 2016). Additionally, it is worth noting that this species can be used as an ornamental plant in urban tree planting for streets, squares, and parks (Araújo e Pires, 2009).

The natural populations of mangabeira trees have been drastically reduced due to the expansion of agricultural and livestock activities, as well as real estate speculation, especially in coastal areas, which are suppressing the native vegetation and, consequently, the natural habitats of this species (Álvares-Carvalho *et al.*, 2022; Oliveira *et al.*, 2014). Another challenge to conserving this species is that its seeds are recalcitrant, making long-term storage unviable (Nunes *et al.*, 2022).

The main source for supplying the mangaba market is extractive activity, and there are only a few orchards established for rational exploitation for fruit production (Soares *et al.*, 2020). According to data from IBGE (2021), Brazil's national production amounted to 2,173 t, with the Northeast region being the largest producer in the country, particularly the states of Paraíba (882 t) and Sergipe (457 t). Therefore, the expansion into new markets is contingent upon the commercial production of mangaba, as the current production does not meet the consumer market's demand (Soares *et al.*, 2020).

The main method for obtaining seedlings of the species is still through seeds, which discourages its cultivation due to slow field growth and uneven fruit production among progenies, thus making it difficult to expand commercial orchards (Ganga *et al.*, 2010; Vieira *et al.*, 2013). Strategies for seedling production through asexual techniques such as micropropagation, grafting, or rooting of cuttings are more promising for the establishment of commercial orchards. Grafting has been successfully applied to botanical varieties from the Cerrado due to the rapid growth of rootstocks (Pereira *et al.*, 2006). Micropropagation protocols have been established by several authors (Oliveira *et al.*, 2016; Prudente *et al.*, 2016; Sá *et al.*, 2012; Soares *et al.*, 2011), but there are no biofactories with large-scale production of seedlings and no protocols for cutting propagation.

A bibliometric review is a statistical approach used to quantify the impact of research by analyzing performance and productivity measures (Romanelli *et al.*, 2021). Additionally, bibliometrics provides insights into the current state of a knowledge area, assisting in the development of future research lines (Chaves Almeida *et al.*, 2022). Thus, this technique can be applied to the topic of propagation and conservation of the mangabeira tree to support future research actions.

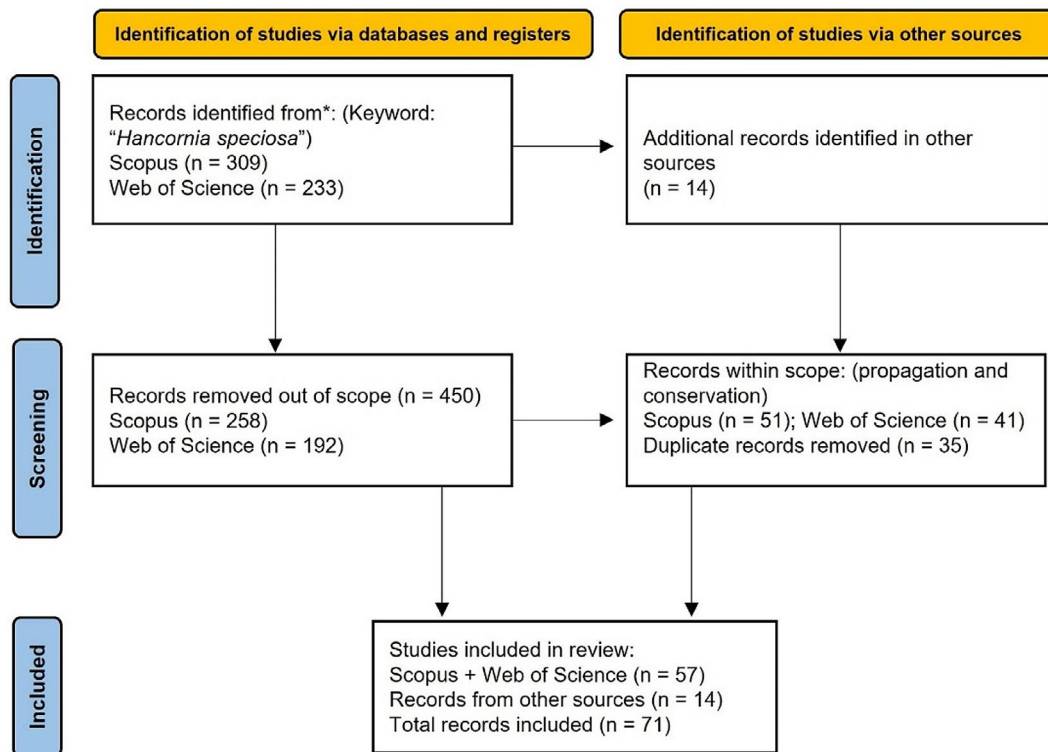
Therefore, this review aims to explore the main studies on the propagation and conservation of the *H. speciosa* tree, including articles related to *in vitro* and *ex vitro* propagation, *in vitro* conservation, and cryopreservation. A mixed methodology of bibliometric analysis and literature review was adopted to present the state of the art and prospects for expanding large-scale sexual and asexual propagation techniques of this native fruit tree.

## METHODS

The present study was based on bibliometric analysis, followed by a systematic literature review (Araújo *et al.*, 2020), following the guidelines of the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) methodological tool (Moher *et al.*, 2009; Page *et al.*, 2021). A bibliographic search was conducted in the following scientific databases: SCOPUS (<http://www.scopus.com>) and Web of Science (<http://www.webofknowledge.com>). The literature search was conducted in March 2023 using the following keyword: "*Hancornia speciosa*." No time interval was specified to gather as much information as possible. Articles published in English, Spanish, and Portuguese were included.

The review consisted of three steps: (i) identification of articles in the Web of Science and Scopus databases that contained specific descriptors, as well as papers from other sources that were not detected by the descriptors but were relevant to this review; (ii) screening (omitting duplicates, reviews, as well as unavailable papers and those outside the scope of the present study); and (iii) inclusion of eligible, full-text articles that were available online and provided information related to mangabeira propagation and conservation.

The search for the keyword "*Hancornia speciosa*" identified 543 documents, of which 309 were on the Scopus platform and 233 were on the Web of Science platform. All these documents had their titles and abstracts read to exclude documents that were not related to the propagation and conservation of mangabeira, of which 51 articles were selected from Scopus and 41 from Web of Science. After that, the duplicates existing in the two platforms were excluded, and another 14 articles considered important for the review and not identified in these platforms were also inserted, forming a total of 71 articles (Figure 1). The quantitative analyses of the bibliometric indexes were compiled in a Microsoft Office Excel® 365 spreadsheet. Finally, VOSviewer (Eck e Waltman, 2010) was used to set up cooperation networks among authors, keywords, and organizations.



**Figure 1.** PRISMA 2020 flow diagram in this systematic review.

## RESULTS AND DISCUSSION

### Years of publication

Bibliographic research on the propagation and conservation of mangabeira revealed that the first work on this species was published in 1992 and dealt with the effect of different levels of humidity on the germination of its seeds (Oliveira e Valio, 1992). Since then, studies have been published almost every subsequent year, until the year 2022. It is important to note that between 2009 and 2022, 57 papers were published, corresponding to approximately 79.1% of the total number of papers published during the entire period evaluated, with an average of about 4 papers published per year (Figure 2).

### Most relevant authors

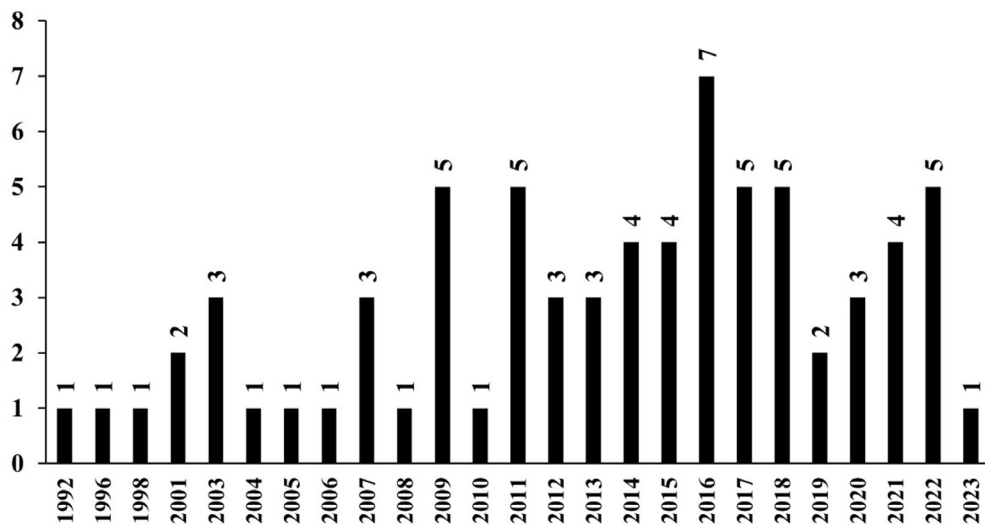
Below is a figure where we can observe the most relevant authors in the field of propagation and conservation of the mangaba tree, who have published works related to this theme (Figure 3). We can highlight that there are four main clusters, in which the main authors are distributed. The red cluster is formed by the research group of Dr. Renato Paiva of the Universidade Federal de Lavras, with nine papers, which together with other researchers has developed work focused mainly on the cryopreservation of mangabeira (Prudente et al., 2017; Prudente et al., 2017; Santos et al., 2015; Santos et al.,

2015). The dark blue cluster is composed of the research group of Dr. Ana da Silva Lédo from Embrapa Tabuleiros Costeiros, with 8 articles, whose work is mainly focused on the propagation and *in vitro* conservation of this species (Lédo et al., 2007, Sá et al., 2011, Sá et al., 2012).

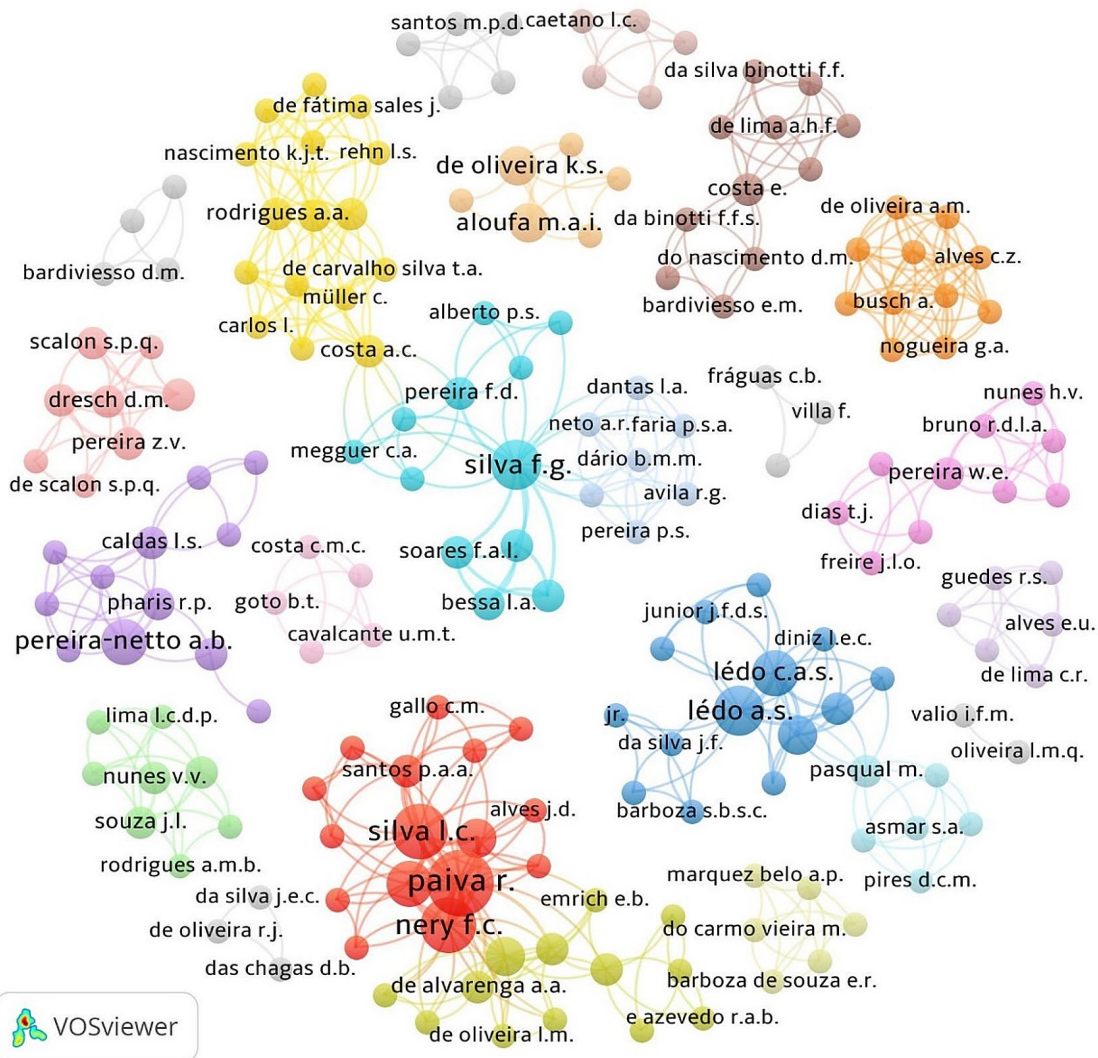
The light blue cluster is formed by researchers from the research group of Dr. Fabiano Guimarães Silva from the Instituto Federal Goiano, Campus Rio Verde, with five papers, whose research is mainly focused on micropropagation and seedling production, with emphasis on the deficiency of macronutrients and micronutrients (Bessa et al., 2012, 2013). Finally, the purple cluster is composed of the research group of Dr. Adauto Bellarmino de Pereira Netto from the Universidade Federal do Paraná, with 5 published papers, which stands out for having started research with the *in vitro* propagation of mangabeira, with the first paper published in 1996 (Pereira-Netto, 1996).

### Research Institutions

The most relevant research institutions are illustrated below, where it can be observed that five institutions concentrate the majority of published works related to the propagation and conservation of the mangabeira tree. The Federal University of Lavras (UFLA), the Federal University of Paraíba (UFPB), the Federal University of Sergipe (UFS), the Brazilian Agricultural Research Corporation (EMBRAPA), and the Federal Institute of Goiano (IFGOIANO) stand out (Figure 4).

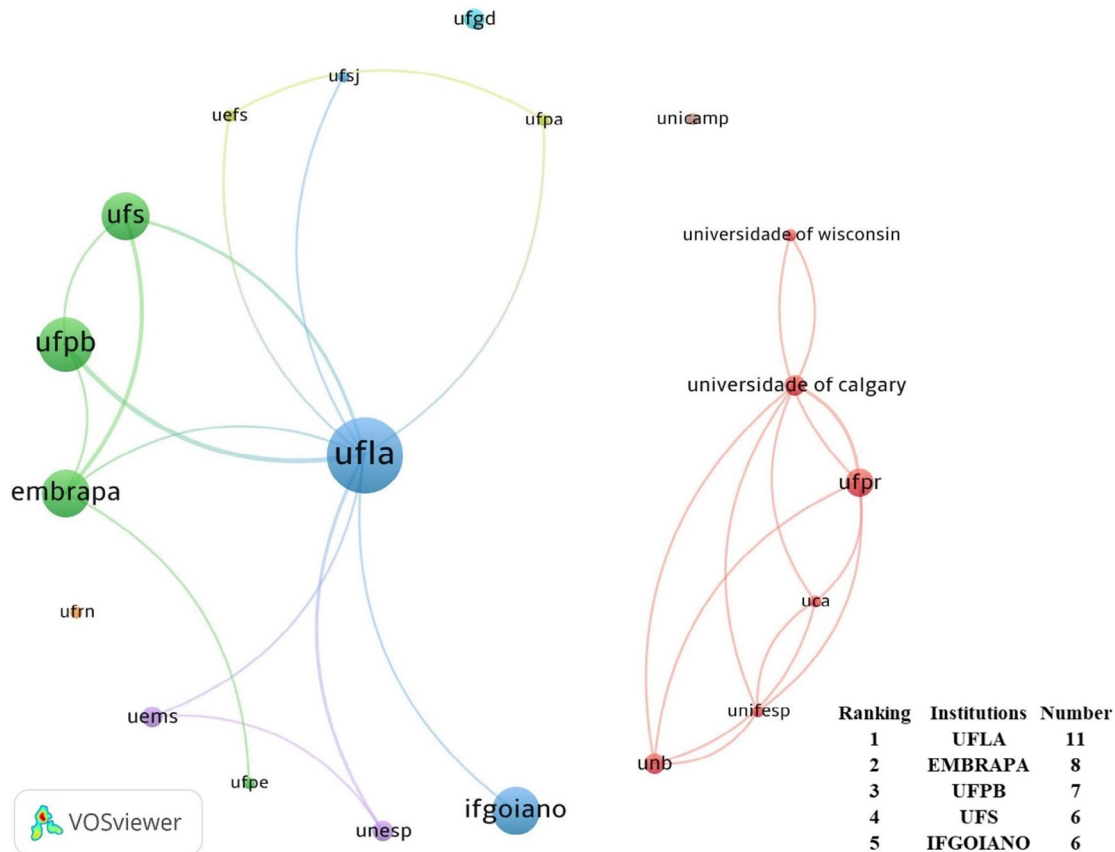


**Figure 2.** Number of articles by year of publication on mangabeira propagation and conservation.



**Figure 3.** Most relevant authors (based on several published papers) on the propagation and conservation of mangabeira.





**Figure 4.** Most relevant research institutions (based on several published papers) on the propagation and conservation of mangabeira. \*UFLA = Universidade Federal de Lavras; UFPB = Universidade Federal da Paraíba; UFS = Universidade Federal de Sergipe; EMBRAPA = Empresa Brasileira de Pesquisa Agropecuária; IFGOIANO = Instituto Federal Goiano; UFRN = Universidade Federal do Rio Grande do Norte; UFPE = Universidade Federal de Pernambuco; UFPR = Universidade Federal do Paraná; UEMS = Universidade Estadual do Mato Grosso do Sul; UNESP = Universidade Estadual Paulista; UEFS = Universidade Estadual de Feira de Santana; UFSJ = Universidade Federal de São João del-Rei; UFGD = Universidade Federal da Grande Dourados; UFPA = Universidade Federal do Pará; UNICAMP = Universidade Estadual de Campinas; UNIFESP = Universidade Federal de São Paulo; UNB = Universidade de Brasília.

### Analysis of keyword trends

In addition to the analyses presented above, a trend study was conducted based on the keywords used by authors in the field of mangabeira tree propagation (Figure 5). It is evident that, alongside keywords emphasizing the scientific name, fruit, and the species' natural range, studies have predominantly centered around plant tissue culture, with a specific emphasis on micropropagation (Oliveira et al., 2016; Prudente et al., 2016). Moreover, other significant terms highlight aspects related to the species' recalcitrant seeds, germination, and research focused on the mineral nutrition of seedlings (Bessa et al., 2013; Santos et al., 2021).

### Seed technology

Considering the information presented in the table below, it is emphasized that a wide range of factors affects the germination of mangabeira seeds, including storage, processing, drying, seed morphology, and biometrics

(Table 1). Biometric analyses of fruits and seeds have revealed significant phenotypic variability among different mangabeira accessions (Soares et al., 2019). Regarding processing, manual extraction proves more effective in obtaining seeds of higher physiological quality compared to mechanical methods (Barros et al., 2006; Nunes et al., 2021).

The ideal temperature for germination of *H. speciosa* seeds has been determined to be between 20-30°C (Oliveira e Valio, 1992). In the guidelines for seed analysis of forest species, it is recommended that germination tests occur at 25°C on a paper roll substrate, with eight replications of 50 seeds (Brazil, 2013). Natural drying of *H. speciosa* seeds in a laboratory environment can impair emergence and initial seedling growth after 48 hours (Santos et al., 2010). In addition, reducing the water content between 20% and 5% reduces the physiological potential of the seeds (Masetto e Scalon, 2014). Mangabeira seeds are also influenced by the depth of sowing and the type of substrate and should be planted one centimeter deep in Cerrado soil with up to 15% washed sand (Oliveira et al., 2018).

Importantly, the germination of *H. speciosa* seeds starts from day 12 and extends until day 41, with an average emergence of 55.4% and average days to emergence of 22.64 (Vieira et al., 2015). Mangabeira seeds are classified as recalcitrant, meaning that they do not tolerate drying and storage (Nunes et al., 2022). Some studies have sought to develop protocols for the conservation of mangabeira seeds for long periods. An example is the work done by Nunes et al. (2022), who observed that mangabeira seeds can be stored for up to 50 days in preservative solutions.

In general, this information is valuable for improving the seedling production of this species, as it provides important information about the factors that affect seed quality and can be used to improve conservation practices and large-scale seedling production of this species.

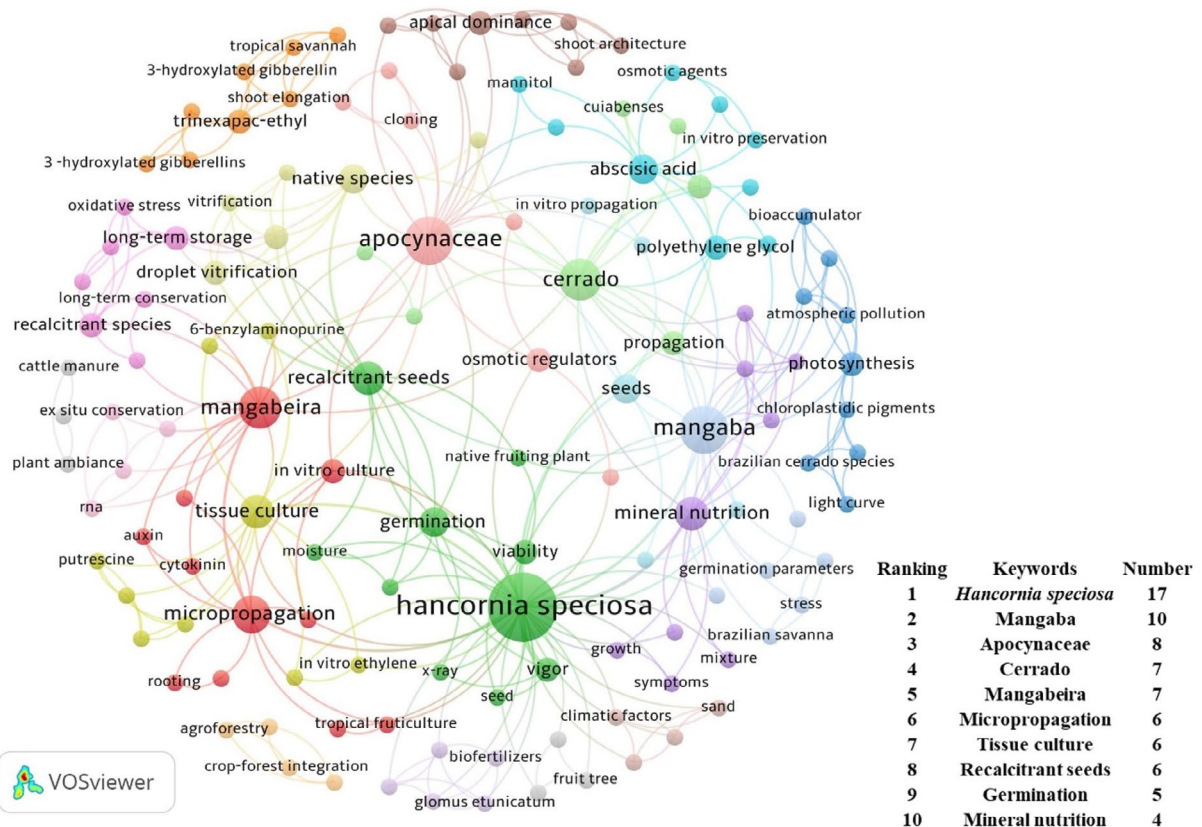
**Production of seedlings**

Studies show that the combination of various materials for substrate composition, such as topsoil, coconut fiber, and sand, can provide a suitable foundation for developing mangabeira seedlings (Dias et al., 2007; Nogueira et al., 2003). The use of bovine manure has also been indicated as an important component for substrate fertilization in the production of seedlings of this species (Arrua et al., 2016; Dias et al., 2009; Oliveira Lima et al., 2020; Silva et al., 2009). It is interesting to note that different

combinations of these materials have been used in various studies, suggesting that the choice of substrate may depend on several factors, such as the type of substrate, botanical variety, and production objectives. This choice stands out as one of the main factors influencing seedling production (Table 2).

Furthermore, there is evidence that inoculation of mycorrhizal fungi can be beneficial for the growth of mangabeira seedlings (Abreu et al., 2022; Cardoso Filho et al., 2008; Costa et al., 2003, 2005). These fungi can form a symbiotic relationship with plant roots, increasing their ability to absorb nutrients from the soil. The choice of the type of mycorrhizal fungus used may depend on soil conditions and nutrient availability (Smith, Read, 2008).

Another important factor in the production of mangabeira seedlings is the adequate nutrition of the plants. The omission of important nutrients, such as N, P, K, Ca, Mg, and S, can result in significant changes in plant growth and the appearance of deficiency symptoms (Bessa et al., 2012, 2013). Therefore, it is critical to ensure that seedlings obtain balanced nutrition, either through substrate or fertilizer application. It is interesting to note that mangabeira can be grown in consortium with other crops, such as cassava. This can bring benefits to both seedling production and final crop production (Martinotto et al., 2012). In addition, the seedlings seem to be able to tolerate water deficits, adapting to stress by increasing root depth and reducing the emission of sprouts (Scalon et al., 2015; Silva et al., 2016).



**Figure 5.** Trends in mangabeira based on authors' keywords on mangabeira propagation and conservation.

**Table 1.** Scientific productions related to mangabeira seeds.

Area	Main result	Reference
Germination	<ul style="list-style-type: none"> <li>• <i>H. speciosa</i> seeds exhibited a higher germination rate when maintained at a temperature between 20-30 °C.</li> <li>• Seedling emergence took place between the 12th and 41st day, with an average emergence rate of 55.4% and an average emergence time of 22.64 days, though variations may exist among different populations.</li> <li>• The depth of sowing and the type of substrate influence the germination of mangabeira seeds.</li> <li>• Planting them 1 cm deep in Cerrado soil with up to 15% washed sand is recommended.</li> </ul>	(Oliveira e Valio 1992; Vieira et al., 2015; Oliveira et al., 2018; Santos et al., 2021)
Storage	<ul style="list-style-type: none"> <li>• Reducing the water content of seeds between 20% and 5% resulted in a decrease in their physiological potential. And natural drying in the laboratory hinders emergence after 48 hours.</li> <li>• <i>H. speciosa</i> seeds can be preserved for up to 50 days in preservative solutions developed for this purpose.</li> </ul>	(Santos et al., 2010; Masetto e Scalon, 2014; Dresch et al., 2016; Nunes et al. 2022c)
Processing	<ul style="list-style-type: none"> <li>• Manual seed extraction resulted in higher physiological quality compared to mechanical extraction.</li> </ul>	(Barros et al., 2006; Nunes et al., 2021)
Biometrics	<ul style="list-style-type: none"> <li>• Biometric analyses of fruits and seeds revealed a significant phenotypic variability among different mangabeira accessions.</li> </ul>	(Soares et al., 2019)

The production of mangabeira seedlings by vegetative propagation, whether by staking, budding, or grafting, is still little explored, especially for the northeastern botanical variety *H. speciosa* var. *speciosa* Gomes, which hinders its cloning on a large scale and, consequently, the establishment of commercial orchards. Regarding staking, the study by Soares et al. (2020) revealed total mortality of the cuttings, but no anatomical impediments were found for the rooting of the cuttings. Vieira et al. (2020) observed a 100% survival of the cuttings, but only with the emission of root primordia. No scientific articles on the propagation of mangabeira by air layering were found in the review. However, in a master's thesis conducted by (Reis, 2011), it was observed that 25% of simple layerings took root, irrespective of the substrate or the concentration of indole butyric acid used. Similarly, (Tiago, 2020) achieved success in layering different varieties of mangabeira. Additionally, no articles were found reporting the propagation of mangabeira by grafting. Nevertheless, this technique is detailed in books and technical communications concerning Cerrado varieties and can be executed through lateral or top grafting and budding. Success rates range from 60% to 90% on 12-month-old rootstocks (Lédo et al., 2015; Pereira et al., 2006).

In summary, the production of mangabeira seedlings involves several important factors, such as the choice of substrate, inoculation of mycorrhizal fungi, adequate nutrition, and adaptation to environmental conditions should be considered. Large-scale vegetative propagation is still not widespread, making it difficult to establish plantations. The knowledge of these factors can contribute to the production of healthy seedlings of high genetic quality, capable of surviving the adverse conditions of the field.

### **In vitro propagation**

Plant tissue culture presents several techniques that can assist in large-scale propagation and *ex-situ*

conservation of native fruit species such as *H. speciosa*. Among these techniques, we can highlight *in vitro* germination, micropropagation, *in vitro* conservation, and cryopreservation. Micropropagation is the technique that has presented the most significant impacts and concrete results within the culture of plant tissue (Abdalla et al., 2022). This technique encompasses several stages, ranging from *in vitro* establishment and germination, through multiplication and rooting to acclimatization (George et al., 2007).

The *in vitro* germination of mangabeira seeds is a fundamental process for the efficient *in vitro* propagation of this species. Several studies have been conducted to identify the factors that influence *in vitro* germination and, consequently, optimize this technique. One of the aspects investigated was the culture medium used. It has been verified that the MS medium, with half of the saline concentration or the WPM medium, supplemented with 2.0 g L<sup>-1</sup> of activated carbon or with 15.0 g L<sup>-1</sup> of sucrose and 0.2 mg L<sup>-1</sup> of GA<sub>3</sub> respectively, promoted high percentages of germination and good development of the aerial part and root system of mangabeira seedlings (Lédo et al., 2007, Soares et al., 2009). Different support agents were also tested, verifying that the presence of vermiculite, either alone or combined with sand, exerted a positive influence on the *in vitro* germination of mangabeira seeds (Oliveira et al., 2014).

Regarding micropropagation, there are several options for culture medium and growth factors that have been tested, as can be seen in Tab. 3. The multiplication of stem apices and nodal segments in an MS medium supplemented with 4 µM of BA (benzyladenine) and 2.5 µM of AIB showed to be an efficient technique for the *in vitro* propagation of mangabeira (Pereira-Netto, 1996). The addition of 1.0 mg L<sup>-1</sup> or 2.0 mg L<sup>-1</sup> in the WPM medium allows well-developed buds to be obtained (Soares et al., 2007, 2011). Moreover, *in vitro*, rooting of *H. speciosa* can be achieved in a WPM medium supplemented with the combination of 4.92 µM ANA and 4.92 µM AIB (Prudente et al., 2016).

**Table 2.** Scientific productions related to the production of mangabeira seedlings.

Area	Main result	Reference
Substrate	<ul style="list-style-type: none"> <li>Plants grown in natural soil demonstrate better adaptation to the environment due to the reduction in diffusive resistance, promoting gas exchange during periods of greater evaporative demand.</li> <li>To achieve maximum fertility, the recommended substrate composition is 46% vegetable soil, 39% coconut fiber, no manure, and 15% sand, with the addition of 11 g L<sup>-1</sup> of triple superphosphate.</li> <li>For optimal growth and nutritional quality of mangabeira seedlings, the suggested substrate is 14% cattle manure, 56% vegetable soil, 15% coconut fiber, 15% sand, and 4 g dm<sup>-3</sup> of triple superphosphate.</li> <li>The best results for developing healthy, high-quality seedlings with good field survival were obtained with substrates D (bovine manure + Plantmax® + soil, in a ratio of 1:1:3) and E (bovine manure + soil, in a 2:3 ratio).</li> </ul>	(Nogueira et al., 2003; Dias et al., 2007; Dias et al., 2009; Silva et al., 2009; Silva et al., 2011)
Environment	<ul style="list-style-type: none"> <li>Both black and aluminized screens are recommended to produce mangabeira seedlings. In addition, it is indicated to grow in shaded environments from 0% to 18%.</li> </ul>	(Arrua et al., 2016; Lima et al., 2020)
Mycorrhizal Fungi	<ul style="list-style-type: none"> <li>Mangabeira seedlings exhibit a positive response to the presence of native arbuscular mycorrhizal fungi in the rhizosphere.</li> <li>These fungi promote increased growth and enhanced phosphorus absorption in the aerial part, particularly in natural soil. This effect reduces the time required for transplanting seedlings from the nursery to the field.</li> <li>Mangabeira is highly dependent on mycorrhizae, although the degree of dependence varies based on phosphorus and fungal inoculum levels.</li> </ul>	(Costa et al., 2003; Costa et al., 2005; Cardoso Filho et al., 2008; Abreu et al., 2022)
Nutrient Deficiency	<ul style="list-style-type: none"> <li>The omission of nutrients such as nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur in the nutrient solution can negatively impact the characteristics of the seedlings.</li> </ul>	(Bessa et al., 2012, 2013)
Water deficit	<ul style="list-style-type: none"> <li>During the water stress period, mangabeira seedlings adopt a main strategy of altering their growth pattern, with increased root depth and reduced shoot emission, to maintain tissue hydration and tolerate severe water deficits.</li> </ul>	(Scalon et al., 2015; Silva et al., 2016)
Cuttings	<ul style="list-style-type: none"> <li>For mangabeira, survival values of 100% were observed in all treatments evaluated. In addition, root primordia were observed in the herbaceous cuttings.</li> <li>Both herbaceous and woody stem cuttings presented total mortality and lack of rooting, independently of the high concentrations of IAB (indol butyric acid) and the origin of the cuttings.</li> </ul>	(Vieira et al., 2020; Soares et al., 2020)
Others	<ul style="list-style-type: none"> <li>The average survival rate of mangabeira in intercropping with cassava was 98%.</li> <li>The average monthly growth rate of mangabeira varied from 0.1 cm to 6.1 cm, resulting in a final average growth rate of 2.19 cm.</li> <li>Exogenous application of aluminum can directly affect seed embryos, preventing seedling establishment.</li> <li>Regardless of the subspecies, the mangabeira presents limitations for establishment in the field, and seedling transplantation is recommended at 90 days of age.</li> <li>The application of gibberellins offers a practical method for nursery production of <i>H. speciosa</i> seedlings for field planting.</li> </ul>	(Caldas et al., 2009; Martinotto et al., 2012; Vieira et al. 2013; Rodrigues et al., 2017; Santos et al., 2018)

These results indicate that mangabeira plants multiplied in flasks sealed with para-film® and PVC film have a higher number of sprouts in both the first and second subcultivation after 65 days (Sá et al., 2012). The use of sealing lids that allow the exchange of gases between the container and the external environment provides an increase in CO<sub>2</sub> concentration in the container and consequently a reduction in ethylene concentration (Fortini et al., 2021). The accumulation of ethylene in the container negatively influences the development of the plant and may cause leaf abscission and apical necrosis, which has been a limiting factor in the *in vitro* propagation of mangabeira (Teixeira da Silva et al., 2020). Other studies have shown that inoculation of bacterial isolates during mangabeira

micropropagation influenced the number of buds and expanded leaves (Cabral et al., 2018). The occurrence of somaclonal variation during micropropagation of *H. speciosa* was also not observed (Costa et al., 2022).

Overall, the table below shows a wide variety of techniques and factors that have been tested for *in vitro* propagation and cultivation of mangabeira (Table 3). The findings suggest that the success of micropropagation, growth, and *in vitro* germination of mangabeira depends on several factors, including culture media, plant growth regulators, temperature, and culture conditions. These results may be useful for future studies on the *in vitro* propagation and cultivation of mangabeira and other plant species.



**Table 3.** Scientific productions focusing on in vitro propagation of mangabeira.

Area	Main result	Reference
In vitro germination	<ul style="list-style-type: none"> <li>• Treatment of mangabeira seeds with 0.1 mg L<sup>-1</sup> of gibberellic acid promotes in vitro germination.</li> <li>• The MS culture medium with half the saline concentration and 2.0 g L<sup>-1</sup> of activated charcoal resulted in 100% germination and robust development of both the aerial part and root system.</li> <li>• The highest percentage of <i>in vitro</i> germination for mangabeira seeds was achieved in WPM and ½ MS culture media, supplemented with 15.0 g L<sup>-1</sup> of sucrose, 0.2 mg L<sup>-1</sup> of GA<sub>3</sub>, and pH adjusted to 5.8.</li> <li>• Mangabeira seeds exhibited the best germination rates when inoculated in a liquid medium either without sucrose or with 15.0 g L<sup>-1</sup> of sucrose.</li> <li>• Media containing vermiculite and its combinations, such as vermiculite + sand (T3), vermiculite + water (T1), and vermiculite + sand (T2), positively influenced the in vitro germination of <i>H. speciosa</i>.</li> <li>• Natural drying of <i>H. speciosa</i> Gomes var. <i>gardneri</i> can be conducted up to 106 hours after fruit extraction (36.8% water content) without adversely affecting the in vitro germination rate.</li> </ul>	(Pinheiro <i>et al.</i> , 2001; Lédo <i>et al.</i> , 2007; Soares <i>et al.</i> , 2009; Cabral <i>et al.</i> , 2014; Oliveira <i>et al.</i> , 2014; Santos <i>et al.</i> , 2017)
Micropropagation	<ul style="list-style-type: none"> <li>• The use of developing axillary buds released by 1-methylcyclopropene is a promising method to increase the in vitro multiplication potential of <i>H. speciosa</i>.</li> <li>• <i>H. speciosa</i> can be propagated vegetatively by multiplying stem apices and nodal segments on MS medium supplemented with 4 µM BA and 2.5 µM IBA.</li> <li>• Concentrations of 1.0 or 2.0 mg L<sup>-1</sup> of BAP made it possible to obtain more developed shoots.</li> <li>• The WPM medium, supplemented with 2.0 mg L<sup>-1</sup> of BAP, in the multiplication phase, induces the best organogenic responses in the cultivation of mangabeira stem segments.</li> <li>• In the establishment phase, the adventitious shoots regenerated in bottles sealed with Para-film® and PVC film showed a greater number of nodes and sprouts.</li> <li>• Micropropagation of <i>H. speciosa</i> did not influence the occurrence of somaclonal variation.</li> </ul>	(Pereira-Netto, 1996; Pereira-Netto, 2001; Machado <i>et al.</i> , 2004; Soares <i>et al.</i> , 2011; Sá <i>et al.</i> , 2012; Oliveira <i>et al.</i> , 2016; Vieira <i>et al.</i> , 2018; Cabral <i>et al.</i> , 2018; Costa <i>et al.</i> , 2022)
Growth in vitro	<ul style="list-style-type: none"> <li>• Gibberellins (GAs) A1, A3, A4, and A7, all 3β-hydroxylated GAs, active in growth, significantly inhibited shoot elongation and knot formation in <i>H. speciosa</i> grown in vitro.</li> <li>• Temperature-induced lateral branch elongation in <i>H. speciosa</i> is related to altered ethylene dynamics. A high growth temperature (35 °C) leads to reduced ethylene evolution and increased lateral branching.</li> <li>• Among the different concentrations of sugars analyzed, sucrose and 3% glucose (30 g L<sup>-1</sup>) improved the accumulation of fresh and dry mass.</li> <li>• The methodology used in this study was able to adequately measure photosynthetic responses in <i>H. speciosa</i> seedlings grown <i>in vitro</i>, indicating a relative functionality of the photosynthetic apparatus of these species under the conditions of this study and exhibiting substantial rates of CO<sub>2</sub> assimilation under high levels of irradiance.</li> </ul>	(Pereira-Netto e McCown, 1999; Pereira-Netto <i>et al.</i> , 2003; Costa <i>et al.</i> , 2014; Dantas <i>et al.</i> , 2021)
Rooting/ Acclimatization	<ul style="list-style-type: none"> <li>• During the acclimation phase, mangabeira seedlings showed better growth of the aerial part, a higher number of leaves, and a higher number of nodes when grown on substrates composed of a mixture of sand and dry coconut shell powder (1:1) or vermiculite and sand (1:1).</li> <li>• <i>H. speciosa</i> can be rooted <i>in vitro</i> in WPM medium supplied with the combination of 4.92 µM ANA and 4.92 µM IBA.</li> <li>• <i>H. speciosa</i> plants from indirect organogenesis rooted <i>in vitro</i> can be acclimatized, showing 100% survival.</li> </ul>	(Freire <i>et al.</i> , 2011; Prudente <i>et al.</i> , 2016)
Callogenesis	<ul style="list-style-type: none"> <li>• The exogenous application of polyamines in <i>H. speciosa</i> does not promote an increase in callus growth.</li> <li>• The largest callus mass was observed in the BI accession, in the presence of 22.62 µM of 2,4-D and 11.10 µM of BA.</li> </ul>	(Fráguas <i>et al.</i> , 2009; Lédo <i>et al.</i> , 2023)

### **In vitro conservation and cryopreservation**

The studies presented in the table below present significant advances in the area of *in vitro* conservation and cryopreservation of mangabeira (Table 4). *In vitro* conservation techniques of nodal segments and mangabeira micro shoots have been studied under different conditions, including the presence or absence of sucrose and sorbitol, as well as the use of abscisic acid in flasks sealed with aluminum foil (Lédo et al., 2011; Sá et al., 2011; Santos et al., 2011). The results indicate that the technique of *in vitro* conservation by slow growth in a half-strength culture medium or supplemented with sucrose and sorbitol is viable for a period of 90 to 120 days and that the use of abscisic acid at a concentration of 0.5 mg L<sup>-1</sup> is efficient for the *in vitro* conservation of mangabeira micro shoots (Lédo et al., 2011, Pires et al., 2019, Oliveira e Aloufa, 2022, Pires et al., 2022). Osmoregulators such as sucrose and sorbitol, depending on the concentration used, perform the function of removing excess intracellular water through an osmotic gradient.

This results in slower growth of the culture, which allows its conservation (El-Bahr et al., 2016; Flores et al., 2013).

Regarding cryopreservation, the drop vitrification and encapsulation-vitrification techniques were successfully tested for mangabeira (Prudente et al., 2017; Santos et al., 2015). In addition, the combination of BA×ANA was used to promote greater regeneration of *H. speciosa* stem apices after cryopreservation (Santos et al., 2015). Other studies explored encapsulation in sodium alginate capsules with BAP and precultivation of lateral buds in medium with proline for 24 hours, which resulted in higher regeneration rates of *H. speciosa* lateral buds (Prudente et al., 2014; Prudente et al., 2017; Prudente et al., 2017). However, limitations in the cryopreservation technique were also found, such as the lack of survival and regeneration of mangabeira zygotic embryos after cryopreservation by the desiccation technique (Santana et al., 2018). In summary, the studies present important advances in the area of *in vitro* conservation and cryopreservation of mangabeira, demonstrating the feasibility of different techniques for the long-term conservation of this species.

**Table 4.** Scientific productions focusing on *in vitro* conservation and cryopreservation of mangabeira.

Area	Main result	Reference
<i>In vitro</i> conservation	<ul style="list-style-type: none"> <li>• Greater proline accumulation occurs in stem segments of mangabeira adventitious shoots cultivated <i>in vitro</i>. Increasing sorbitol concentration does not induce an increase in proline synthesis in mangabeira stem and leaf segments.</li> <li>• For medium-term conservation of mangabeira native to the Brazilian Cerrado, it is possible to use the slow growth technique for up to 90 days in WPM medium at 20 °C.</li> <li>• The <i>in vitro</i> conservation of <i>H. speciosa</i> microplants under slow growth conditions for 120 days is feasible in MS medium supplemented with 15 g L<sup>-1</sup> of sucrose and 5 g L<sup>-1</sup> of sorbitol.</li> <li>• The best results for plant conservation are obtained by adding 30 g L<sup>-1</sup> of sucrose to the cultivation medium and carrying out <i>in vitro</i> conservation for up to 90 days.</li> </ul>	(Santos et al., 2011; Santos et al., 2016; Pires et al., 2020; Oliveira & Aloufa, 2022; Pires et al., 2022)
Cryopreservation	<ul style="list-style-type: none"> <li>• Cryopreservation of <i>H. speciosa</i> stems can be successfully conducted after loading for at least 20 minutes before PVS2 treatment. The BA × ANA combination promotes greater regeneration of <i>H. speciosa</i> shoot tips.</li> <li>• Encapsulation-vitrification proves efficient for long-term conservation, yielding high survival rates following the cryopreservation of <i>H. speciosa</i> lateral buds. Sodium alginate capsules with 0.2 μM BAP enhance regeneration, resulting in longer shoots and a greater number of leaves.</li> <li>• The oxidative stress induced by cryopreservation is significantly reduced by pre-cultivating lateral buds in 0.1 M proline for 24 hours, promoting greater shoot regeneration.</li> <li>• Encapsulation-vitrification remains efficient for long-term conservation, ensuring high survival rates after cryopreservation of <i>H. speciosa</i> lateral buds.</li> <li>• The desiccation technique does not support the survival and regeneration of mangabeira zygotic embryos after cryopreservation.</li> <li>• Successful cryopreservation of various mangabeira accessions was achieved using the droplet vitrification technique with different exposure times to PVS2.</li> </ul>	(Lédo et al., 2011; Sá et al., 2011; Prudente et al., 2014; Santos et al., 2015; Santos et al., 2015; Prudente et al., 2017; Prudente et al., 2017; Santana et al., 2018; Santana et al., 2022)

## CONCLUSIONS

From the information presented above, it is possible to notice an increase in the studies related to the propagation and conservation of mangabeira in the last ten years, showing its relevance. Moreover, it is noticeable the advance of scientific knowledge about this species in several areas of study, especially about seed technology, seedling production, cryopreservation, propagation, and *in vitro* conservation. Linked to the points above, it is possible to consider that the asexual propagation of mangabeira via cutting, or another form of cloning, may become an important subject of study in the coming years since currently there are no well-established protocols for clonal propagation *ex vitro* of this species, which limits the implementation of commercial plantations and highlights the need for research in this area. Furthermore, improving techniques for cryopreservation and storage of mangabeira seeds, and the development of protocols for somatic embryogenesis, maybe a future trend to support research for this species.

## AUTHORSHIP CONTRIBUTION

Project idea: AVSN

Database: AVSN, PAAS, AVCSM, ASL

Processing: AVSN, PAAS, AVCSM, ASL

Analysis: AVSN, PAAS, AVCSM, ASL

Writing: AVSN, PAAS, AVCSM, ASL

Review: AVSN, PAAS, AVCSM, ASL

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