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Effects of droplet vitrification on the regeneration of apical shoot tips of mangabeira accessions

Efeitos da vitrificação em gotas na regeneração de ápices caulinares de acessos de mangabeira

Efectos de la vitrificación por gotitas en la regeneración de ápices de brotes de accesiones de mangabeira

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Abstract
The mangabeira (*Hancornia speciosa* Gomes) is a fruit tree of high potential that is distributed in several regions of the Brazil, but which is of great social economic importance for the Northeast region. Due to the wide possibility of using its fruits that can be consumed in natura or industrialized, and that represent a financial source for traditional communities that practice the extractivism of this species. The objective of this study was to evaluate the efficiency of the droplet vitrification technique for the long-term conservation of five mangabeira accessions, as well as to verify if there is any different behavior among the accessions to the possible effects of the cryopreservation process. The apical shoot tips were subjected to different periods (30 and 50 min for test I; 25, 50 and 75 min for test II) of exposure to plant vitrification solution 2 (PVS2) before plunging into liquid nitrogen. To evaluate the effects of cryoprotective solutions and exposed times on apical shoot tips exposed or not exposed to liquid nitrogen, the percentage of regeneration were observed at 30^{th} and 60^{th} day of *in vitro* cultivation. It was possible to cryopreserve the apical shoot tips from five accessions studied through the droplet vitrification technique. Adjusts must be made in the droplet vitrification technique steps in the pre-culture and post-culture media so that there is a mitigation of the damage caused during the cryopreservation.
Keywords: *In vitro* conservation; *Hancornia speciosa* Gomes; Cryopreservation.

Resumo
A mangabeira (*Hancornia speciosa* Gomes) é uma frutífera de alto potencial que está distribuída em diversas regiões do país, mas que possui grande importância para a região Nordeste. Devido à ampla possibilidade de uso dos seus frutos que podem ser consumidos *in natura* ou industrializados, e que representam uma fonte de renda para famílias que praticam a atividade extrativista dessa espécie. O objetivo deste estudo foi avaliar a eficiência da técnica vitrificação em gotas para a conservação em longo prazo de cinco acessos de mangabeira, como também verificar se há algum tipo de suscetibilidade entre os acessos aos possíveis efeitos do processo de criopreservação. Os ápices caulinares foram submetidos a diferentes períodos (30 e 50 min para o ensaio I; 25, 50 e 75 min para o ensaio II) de exposição à solução de vitrificação vegetal 2 (PVS2) antes da imersão em nitrogênio líquido. Para avaliar os efeitos de soluções crioprotetoras e tempos de exposição em pontas apicais expostas ou não ao nitrogênio líquido, foram observadas as porcentagens de regeneração aos 30 e 60 dias de cultivo *in vitro*. Foi possível criopreservar os acessos estudados através da técnica de vitrificação em gotas. Devem ser realizados ajustes na técnica de vitrificação em gotas

nas etapas de pré-cultivo e nos meios de pós-cultivo para que haja uma mitigação dos danos causados durante o processo de criopreservação.
Palavras-chave: Conservação *in vitro*; *Hancornia speciosa* Gomes; Criopreservação.

Resumen
La mangabeira (*Hancornia speciosa* Gomes) es un frutal de alto potencial que se distribuye en varias regiones del país, pero que es de gran importancia para la región Nordeste. Debido a la amplia posibilidad de aprovechamiento de sus frutos que pueden ser consumidos in natura o industrializados, y que representan una fuente de ingresos para las familias que practican la actividad extractiva de esta especie. El objetivo de este estudio fue evaluar la eficiencia de la técnica de vitrificación por gota para la conservación a largo plazo de cinco accesiones de mangabeira, así como verificar si existe algún tipo de susceptibilidad entre las accesiones a los posibles efectos del proceso de criopreservación. Los ápices de los brotes se sometieron a diferentes períodos (30 y 50 min para el ensayo I; 25, 50 y 75 min para el ensayo II) de exposición a la solución de vitrificación vegetal 2 (PVS2) antes de la inmersión en nitrógeno líquido. Para evaluar los efectos de las soluciones crioprotectoras y los tiempos de exposición sobre puntas apicales expuestas o no a nitrógeno líquido, se observaron los porcentajes de regeneración a los 30 y 60 días de cultivo *in vitro*. Se logró criopreservar las accesiones estudiadas mediante la técnica de vitrificación por gota. Se deben realizar ajustes en la técnica de vitrificación por gotitas en las etapas de precultivo y en los medios de postcultivo para que haya una mitigación del daño ocasionado durante el proceso de criopreservación.
Palabras clave: Conservación *in vitro*; *Hancornia speciosa* Gomes; Crioconservación.

1. Introduction

Hancornia speciosa Gomes is a fruitful species native from Brazil that can be found in several regions, being very well-adapted to areas of open vegetation and present in the Cerrado, Caatinga, Atlantic Forest, Amazon and Pantanal biomes. Its fruit known as mangaba presents itself as an important source of food with high nutritional quality and can be consumed both in natural and processed. The products obtained from mangaba processing have added value and can be of different types such as juices, liqueurs, jellies, and sweets (Freitas de Lima, et al., 2021).

Its production comes from extractivism in areas of forest remnants, where the fruits are harvested mainly by traditional local communities, as well as from some commercial plantations (Nunes et al., 2022a, 2022b). The importance of the mangaba fruit does not only stem from its nutritional value, but also its importance in socioeconomic and environmental value, contributing to the generation of income for several families as well as to the conservation of the biomes in which it is inserted (Paula et al., 2019).

Due to its great economic importance, wide natural distribution, and mainly because it is under intense anthropic pressure, it is fundamental to know the genetic diversity and the structure of the remaining mangabeira populations to elaborate an efficient in situ ad ex situ conservation strategies (Costa et al. , 2015).

According to the Brazilian germplasm resources information network there are 1,428 accessions distributed in eight germplasm banks maintained by public institutions: Embrapa Amapá (86 accessions), Embrapa Cerrados (15 accessions), Embrapa Meio Norte (39 accessions), Embrapa Tabuleiros Costeiros (271 accessions), State Agricultural Research Corporation of Paraíba (540 accessions), Federal University of Alagoas (20 accessions), Federal University of Goiás (57 accessions) and State University of Goiás (400 accessions) (Silva Júnior et al., 2018).

Several field collections may still be susceptible to natural disasters, including those caused by climate change. In addition, plant genetic resources are also exposed to adversities that may be due to a lack of funding and/or poor management (FAO, 2022). Given the vulnerability that accessions kept in BAGs may be exposed, *in vitro* cultivation techniques for conservation are considered opportune. This method is widely applied to species with orthodox, intermediate, and recalcitrant seeds, being a promising alternative to maintain a large number of accessions in a reduced physical space and protected from attacks by diseases, pests, and/or catastrophes (Morais, et al., 2022).

The application of tissue culture techniques for plant conservation is considered a complementary strategy to the conservation of existing genetic variability and allows acceleration of the multiplication of promising genotypes (Pilatti et al.,

2011). Cryopreservation is one of the adopted methods that consist of cryogenic preservation in liquid nitrogen (-196°C or close to it). It is a useful tool for storing plant cells, tissues, and organs at ultra-low temperatures, to ensure the viability and genetic integrity of plant material for a long period (Prudente et al., 2019).

The shoots obtained and maintained *in vitro* are an excellent explant for cryopreservation and also have potential characteristics for micropropagation and genetic stability. The meristematic zone of the shoot tips is composed of a relatively homogeneous population of small, actively dividing cells that have small vacuoles and a high nucleocytoplasmic ratio. These characteristics promote a greater resistance to dehydration compared to highly vacuolated and differentiated cells (Tanaka; et al., 2018).

It is important to note that despite being widely used as an alternative for the conservation of several species, cryopreservation projects must have clear objectives, long-term funding, qualified technical support personnel, the necessary infrastructure, and well-defined protocols so that they can be implemented routinely in plant cryobanks and help establish backup collections of plant genetic resources (Ochatt et al., 2021). The literature reports some cryopreservation protocols in mangabeira, but not all showed satisfactory results (Sartor et al., 2012; Santos et al., 2015; Prudente et al., 2017a; Prudente et al., 2017b; Santana et al., 2018).

Hancornia speciosa Gomes is a species that requires further research, particularly related to floral biology, ex situ conservation, and cryopreservation. Cryopreservation studies used only explants from natural populations, which reinforces that the lack of information on the behavior of different accessions is scarce. Thus, the objective of this study was to evaluate the efficiency of the droplet vitrification technique for the long-term conservation of five accessions of *Hancornia speciosa* Gomes, as well as to verify if there is any different behavior among the accessions in response of the possible cryopreservation effects.

2. Methodology

2.1 Collection, asepsis, and isolation of plant material

To establish the mangaba aseptic seedlings, ripe fruits from five accessions were collected (Table 1.) at the Mangaba Active Germplasm Bank (BAG Mangaba) of Embrapa Tabuleiros Costeiros, municipality of Itaporanga d'Ajuda, Sergipe, Brazil. The seeds were extracted by manual pulping and placed to dry at room temperature for 24 hours under paper towels, to remove excess water. After this step, the seeds were submitted to asepsis, washed in Tween 20® and sterile water to completely remove the pulp, and then immersed in 70% alcohol for 2 minutes, and then in a sodium hypochlorite solution (2-2.5%) of active chlorine for 15 minutes (Passos & Passos, 2004). Subsequently, the seeds were washed five times with sterile water and kept on paper towels for 24 hours at room temperature.

To obtain aseptic seedlings, the seeds were germinated in Wood Plant Medium - WPM (Lloyd & McCown, 1980) with 30 $g.L^{-1}$ of sucrose and gelled with 3 $g.L^{-1}$ of Phytagel®. The pH of the culture medium was adjusted to 5.8 ± 0.1 and previously autoclaved for 15 minutes at 121 ± 1°C and pressure of 1.05 atm. The explants were kept in a growth room for 24 hours at 25± 2 °C, in the presence of light (1 lumen/m^2) and average relative humidity of around 70%.

Table 1 - Identification of accessions used in the Active Mangaba Germplasm Bank (BAGMangaba) of Embrapa Tabuleiros Costeiros.

State	County	Code/Accession
Pará	Salvaterra (Ilha de Marajó)	BAGMangaba 7 – Água Boa - (AB)
Alagoas	Japaratinga	BAGMangaba 19 – Japaratinga - (JA)
Pernambuco	Ipojuca	BAGMangaba 18 – Oiteiro - (OI)
Paraíba	João Pessoa	BAGMangaba 11 – Paratibe - (PA)
Sergipe	Indiaroba	BAGMangaba 4 – Terra Caída - (TC)

Source: This table was developed by the authors.

2.2 Preculture

From aseptic seedlings grown *in vitro* (for three months), the apical shoot tips (approximately 1.0 mm^2) were excised and pre-cultured in sterile polystyrene Petri dishes containing WPM culture medium supplemented 0.3 M sucrose and gelled with 3g.L^{-1} of Phytagel®. The pH of the culture medium was adjusted to 5.8 ± 0.1 and previously autoclaved according Soares et al. (2009). The explants were kept in a growth room for 24-h at 25± 2 °C, in the presence of light (1 lumen/m^2) and average relative humidity of around 70%.

2.3 Cryoprotection and cryopreservation

After the preculture step, the explants were treated for 20 minutes with a Loading Solution composed of 2 M glycerol and 0.4 M sucrose dissolved in MS medium (Murashige & Skoog, 1962) at room temperature, and then immersed in Vitrification Solution for Plant 2 - PVS2 (SAKAI et al., 1990): (30% (v/v) Glycerol; 15% (v/v) Ethylene Glycol and 15% (v/v) Dimethylsulfoxide-DMSO.

For exposure to the cryoprotective solution, with the aid of a Pauster pipette, drops of approximately 0.25 mL of PVS2 were aspirated and deposited on aluminum paper strips on an ice plate. The explants were immersed in a drops and kept for the following time's exposure time: 30 and 60 minutes (Test I for AB, OI and PA accessions); 25, 50 and 75 minutes (Test II for JA and TC accessions) at 0°C. After each exposure time, the strips containing the explants were immersed in liquid nitrogen (-196°C) and inserted into sterile polystyrene cryotubes, where they remained for 30 minutes.

2.4 Reheating, post-cultivation, and regeneration

After droplet vitrification, the apical shoot tips were reheated in an unloading solution composed of the MS culture medium, supplemented with 1.2 M sucrose at room temperature of 25±2 °C for 15 minutes. Then, the explants were cultured in sterile polystyrene Petri dishes containing WPM post-culture medium, supplemented with 0.3 M sucrose, 0.02 g.L-1 of ascorbic acid and 3 g.L-1 of Phytagel® for 24 hours , in the absence of light. After 24 hours, the medium was replaced by a WPM regeneration medium, supplemented with 0.09 M of sucrose, 0.002 g.L-1 of BAP, 0.02 g.L-1 of ascorbic acid and 3 g.L-1 of Phytagel®.

The cultures were kept in a growth room with a controlled temperature of 25±2°C, and average relative humidity of around 70%, in the absence of light for six days to avoid oxidation, when they were then transferred to exposure to indirect light – 1439 lumens/m^2. To evaluate the effects of cryoprotective solutions and exposed times on apical shoot tips exposed (LN+) or not exposed (LN-) to liquid nitrogen, the percentage of regeneration were observed at 30^{th} and 60^{th} day of *in vitro* cultivation. For the evaluation of the regeneration, a scale was established for the qualitative characteristics observed in the explants, as described in Table 2.

Table 2 - Classification (scale) of shoot tips characteristics observed after cryopreservation during regeneration

Scale	Observed characteristic of the explant
0	White color – Dead Explant
1	Brown color – Oxidized Explant
2	Green color – Regenerated Explant

Source: This table was developed by the authors.

Figure 1 - Apical shoot tips of *Hancornia speciosa* Gomes. Where 0: White color – dead explant; 1: Brown color – oxidized explant 2: Green color–survivor explant.

Color of apical shoot tips of *Hancornia speciosa* Gomes after the droplet vitrification. Source: Photos by the authors

In order to establish the controls (LN-) for each treatment, three apical shoot tips per replicate were submitted to all stages of vitrification in drops, except for storage in liquid nitrogen, to evaluate the possible effects of exposure to the vitrification solution. The regeneration of explants was observed at 30th and 60th days of cultivation after warming.

2.5 Experimental design and statistical analysis

The experimental design for the 2 experiments was completely randomized in a factorial scheme. The first consisted of a 3 x 2 scheme (three accessions: AB, OI, and PA x two exposure times: 30 and 50 minutes), and the second in a 2 x 3 scheme (two accessions: JA and TC x three exposure times; 25, 50 and 75 minutes), with five replicates per treatment, with each replicate consisting of seven explants. The data obtained from the regeneration (scale) were compared by non-parametric Kruskal-Wallis and Mann-Whitney U tests at 5% significance using the R statistical program.

3. Results and Discussion

For experiment I, there were no significant difference between the mangaba accessions, exposure time to PVS2 and interaction between the studied factors. Comparing the percentage of occurrence scale with the control (LN-) treatments, it was possible observed that the AB accession presented 100% of survivor explants at 30th day of cultivation when submmite to 30 min of PVS2 (Figure 2A). Probably the PVS2 solution was not toxic and deleterious to the AB accession at least during this exposure time. All other accessions studied did not differ statistically from their controls, this behavior indicates that even after exposure to cryoprotective solution and storage in liquid nitrogen, the explants maintained a regeneration performance similar to that obtained from their controls (No exposure to liquid nitrogen).

The cryoprotectors present in vitrification solutions play key roles during the application of cryopreservation techniques by enabling cells to be prepared for storage at ultra-low temperatures and to be recovered with high levels of

functionality. These solutions are applied in the molar concentration range, with multiple actions combining specific and non-specific targets, being divided into permeable and non-permeable agents. In most cases, the potential for adverse reactions is linked to permeable agents due to their ability to directly interact with a multitude of cellular activities such as enzymatic processes, transport mechanisms, ion exchanges, and the like (Elliott, et al., 2017).

These adverse effects of exposure to PVS2 could be observed in the other accessions in function of exposure times (Figures 2B, 2C, 2D, 2E and 2F). All treatments, except AB accession combined with 30 minutes PVS2, showed dead and oxidized apical shoot tips when compared to their controls (LN-). However, there was no statistical difference obtained between them, which indicates that the performance of the vitrification solution in the exposure times established for most treatments allowed a similar behavior of the material used even after exposure to liquid nitrogen during the cryopreservation process.

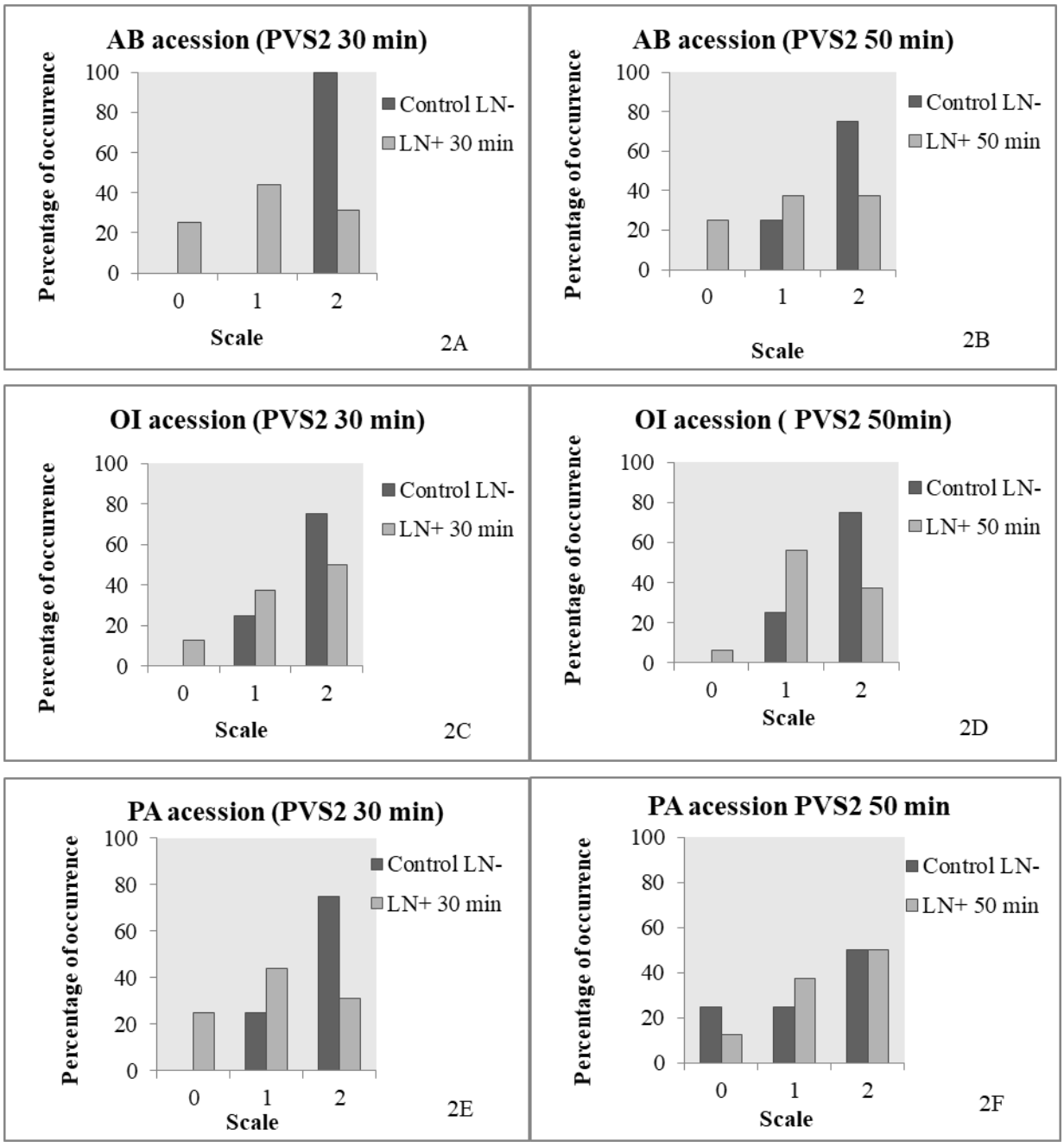
The solutes of cryoprotective agents have a wide range of metabolic and biophysical effects that are necessary for their modes of action, but which can affect cellular biological function. Their protective effects can be considered conflicting due to the toxicity resulting from the use of these solutes, which are often required in molar concentrations, often being above the levels found in normal metabolism. Their biophysical properties allow them to facilitate different cryogenic storage approaches, including vitrification. In this process, the vitrification condition for water depends on both the solute concentrations and the cooling rate of the system. Furthermore, if small ice crystals form during cooling, they can grow large enough to cause severe cell damage during subsequent heating (Tanaka, et al., 2018; Elliott, et al., 2017).

The most common cryoprotective agents used in protocols are compounds of synthetic origin that have different levels of biocompatibility that can cause reactions both in cellular physiology and in the environment. At the cellular level, its toxicity depends on different mechanisms, including membrane effects, alterations in cellular permeability, and interactions with cellular proteins and mitochondrial function. Several strategies have been proposed to minimize the toxicity of cryoprotective agents, including the combination of chemical agents with different levels of toxicity with less toxic or neutralizing cryoprotective agents (Marcantonini et al., 2022), as well as the combination of cryoprotective agents and antioxidants to reduce cell damage caused by osmotic dehydration, the formation of large ice crystals and the incidence of reactive oxygen species (ROS) during cooling and rewarming, thus improving the efficiency of cryopreservation (Liu et al., 2021).

In the case of mangabeira, a protocol using vitrification and drop vitrification techniques were successfully established. According to Santos et al. (2015), the pre-cultivation of shoot tips for 24 hours in a medium with 0.3 M sucrose promotes increased regeneration of shoot tips cryopreserved through both methods of cryopreservation. The authors also found that a period of exposure to PVS2 of 60 minutes promotes significant response differences between the techniques. However, both cryopreservation methods allowed a regeneration rate of more than 70%, where the use of a pre-culture step provided an increase in the regeneration rate of cryopreserved shoot tips.

Stress mitigation during the cultivation process before and after vitrification can further improve cryopreservation technology, obtaining more satisfactory results, as well as reducing the incidence of genetic and epigenetic variations (Wang et al., 2021). Optimization of component concentrations, pre-culture duration, and culture conditions are important factors in improving tolerance to the dehydration process. These factors may vary between species and cryopreservation methods (Bettoni, et al., 2021). The post-cryopreservation treatments are as important as the pre-cooling steps to obtain a satisfactory regeneration. Reheating is carried out at room temperature (as in the case of the drop vitrification technique) or quickly in a water bath at 35-40°C to avoid ice recrystallization, that is, the aggregation of smaller crystals into larger ones (Roque-Borda et al., 2021).

Figure 2 - Percentages of apical shoot tip scale submitted (LN +) and no submitted (LN-, Control) to cryopreservation at 30thday of cultivation in function of times of exposure of PVS2 solution (30 and 50 minutes). Accessions Água Boa (AB), Oiteiro (OI) and Paratibe (PA). Scale 0: Dead explant; 1: Oxidized explant; 2: Survivor explant.

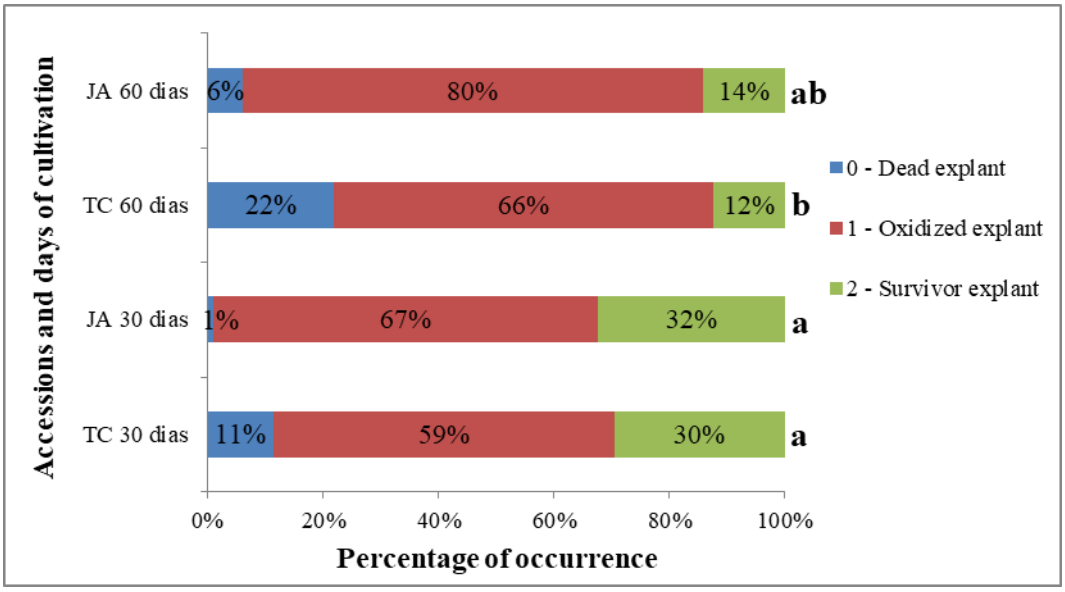


Accessions Água Boa (AB), Oiteiro (OI) and Paratibe (PA). Scale 0: Dead explant; 1: Oxidized explant; 2: Survivor explant. Source: This figure was developed by the authors.

For experiment II, the time of exposure to PVS2 (25, 50, and 75 minutes) was also not significant. However, there was a significant interaction between the Accessions x exposure times. There was no significant difference for accessions at 30th day of cultivation, However ay 60th day of cultivation the accessions presented significant differences. It was possible to observe that the Japaratinga accession (JA) presented a lower percentage of dead explants and higher percentage of survivor explants (Figure 3) when compared to the Terra Caída accession (TC) at 30th and 60th day of cultivation. However, regarding the number of oxidized explants, the JA accession showed a greater susceptibility to the possible effects triggered by oxidative stress during the droplet vitrification process 67 and 80% at 30th and 60th day of cultivation, respectively. According to Ochatt

et al. (2021), oxidative stress induced by cryopreservation disrupts intracellular homeostasis and the balance between the production and elimination of reactive oxygen species (ROS). As antioxidant systems affect the success of cryopreservation, monitoring the activity of key enzymes can help to understand plant metabolism during cryopreservation, thus improving the recovery of cryopreserved plant material.

Figure 3 - Percentages of apical shoot tips according to the regeneration scale obtained at 30 and 60 days of evaluation after droplet vitrification of Accessions Japaratinga and Terra Caída. Scale 0: Dead explant; 1: Oxidized explant; 2: Survivor explant. Means followed by the same letter do not differ by the Kruskal-Wallis test at 5% significance.



Accessions Japaratinga (JA) and Terra Caída (TC). Scale 0: Dead explant; 1: Oxidized explant; 2: Survivor explant. Source: This figure was developed by the authors.

Reactive oxygen species (ROS) produced at low temperatures can cause lipid peroxidation, protein oxidation, and DNA damage that affect cell structure and function, potentially causing cell apoptosis. Traditional cryoprotective agents may often not be sufficient to prevent the incidence of ROS. Thus, the use of antioxidants in pre- and post-culture media can decrease oxidative stress, reduce ROS production, convert ROS into harmless substances and increase the activity of antioxidant enzymes. Therefore, the combined use of antioxidants and cryoprotective agents in cryopreservation can increase the survival rate and reproductive capacity of cells, decreasing osmotic and mechanical damage by ice, thus increasing the efficiency of cryopreservation. However, the use of antioxidants must be done appropriately, especially by observing the concentration in which they are used (Liu et al., 2021).

In the present study, ascorbic acid (ASA) at 0.02 $g.L^{-1}$ concentration was used in post-culture media as an antioxidant to inhibit or reduce possible effects of oxidative stress during and after the application of the drop vitrification technique. According to Ren et al. (2021), treatments with ascorbic acid can considerably reduce ROS levels in cryopreservation steps, including 1st preculture, 2nd preculture, osmoprotectant, and dehydration. However, ASA can also function as a stressor.

It is important to point out that even though a pre-culture step was performed before cryopreservation and the use of antioxidants in the post-culture media for regeneration as in other efficient protocols with mangabeira; even so, high levels of oxidized explants were observed and this number increased with the evaluation time. Such behavior suggests that it is necessary to adjust the concentration of the components used in the culture media, and that oxidative stress influenced the obtained regeneration rates. When evaluating the effect of mangabeira desiccation time on moisture and seed regeneration using cryopreserved embryos, Santana et al. (2018), did not obtain results with any type of regeneration, which demonstrates

that protocols must still be adapted and adjusted according to the cryopreservation technique chosen and the type of explant used.

According to Kaviani and Kulus (2022), cryopreservation is a complementary method to other in situ and ex-situ conservation strategies, but it can still have disadvantages such as: not being efficient with all types of plant material, therefore, cryopreservation protocols for many plant species are not available; the crystallization of ice inside cells that can damage organelles, while cellular dehydration can induce stress; the high intracellular concentration of solutes can be very harmful to cells, as well as cryoprotectants that can affect their viability; successful cryogenics is currently most successful on very small organs and structures, and this is still a constraint; finally, the physiological state of the donor plant material is of great importance.

The cryopreservation of mangabeira shoot tips was established in a viable way through the techniques of vitrification, droplet vitrification, and encapsulation-vitrification (Santos et al., 2015; Prudente et al., 2017a; Prudente et al., 2017b) and can be used for the long-term storage of the genetic resources of that species. However, these studies used explants from natural populations of *Hancornia speciosa* Gomes. This demonstrates a scarcity of information about possible behavioral responses or genetic effects that are triggered individually by different accessions after cryopreservation. Thus, further studies using materials from the Active Mangaba Germplasm Bank should be carried out to verify other types of susceptibility or even tolerance of certain genotypes to the effects of cryopreservation techniques.

It is also important to emphasize that for the efficient reproducibility of protocols, cryopreservation methods must have clear objectives, long-term funding, technical support personnel, adequate infrastructure, as well as well-defined procedures. It is important to highlight that the process of identifying a protocol, testing it, and implementing it for conservation purposes at an applied scale can take years. Often, many factors must be considered and adjusted before implementing a cryopreservation procedure (Bettoni, et al., 2021).

Considering all the factors found in this study, adjustments must be made to the droplet vitrification technique in the pre-culture stages and the post-culture media so that there is a mitigation of the damage caused during the cryopreservation process and, consequently, an increase in the regeneration percentages of shoot tips of accessions of *Hancornia speciosa* Gomes.

4. Conclusion

It was possible to cryopreserve the following mangaba accessions: Água Boa, Japaratinga, Oiteiro, Paratibe, and Terra Caída through the droplet vitrification technique with different exposure times of PVS2.

Adjustments in the concentration of post-cultivation media components must be considered to avoid oxidation and obtain higher explant regeneration rates.

Considering that this is the first study carried out with accessions of Hancornia speciosa Gomes, the data obtained will serve as support for future studies based on the elucidated factors that affected cryopreservation.

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