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CRYOPRESERVATION OF SEEDS OF THE BRAZILIAN NATIVE SPECIES AROEIRA-DO-SERTÃO (*Astronium urundeuva* M. Allemão Engl.)[1]

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ABSTRACT - Aroeira-do-sertão is a Brazilian native species that has been widely explored. Thus, the population of this species has been reduced and techniques for its preservation are essential, such as the conservation of seeds in liquid nitrogen (LN). The objective of this work was to evaluate different cryoprotectant solutions for cryopreservation of aroeira-do-sertão seeds in LN (-196 °C). The treatments used were: control (7.5±1.5 °C); LN without cryoprotectant; sucrose 0.4 mol L^{-1}; sucrose 0.8 mol L^{-1}; glycerol 1 mol L^{-1}; glycerol 2 mol L^{-1}; PVS_1 (plant vitrification solution); PVS_2; PVS_2 + 1% phloroglucinol; and PVS_3. The seeds remained frozen for 120 days. The seeds were evaluated for germination and water content before cryopreservation. Several germination parameters were evaluated on the seventh day and plant growth variables were evaluated after 150 days. The seeds presented 9.2% water content and 74% germination before cryopreservation. The germination in the control treatment was 55%, whereas it varied from 61% to 69% under cryopreservation, denoting the positive effect of cryopreservation, even without cryoprotectants (69%). The seeds presented a triphasic water absorption model; the LN accelerated the germination, which started within 56 hours, whereas the germination in the control treatment started after 66 hours. The plant parts presented satisfactory development after 150 days, as shown by the Dickson quality index. The use of cryoprotectants did not affect seed germination and initial growth of seedlings. Aroeira-do-sertão seeds with 9.2% water content can be cryopreserved in LN without cryoprotectants.

Keywords: Seeds conservation. Liquid nitrogen. Tree species.

CRIOPRESERVAÇÃO DE SEMENTES DA ESPÉCIE NATIVA DO BRASIL AROEIRA-DO-SERTÃO (*Astronium urundeuva* M. Allemão Engl.)

RESUMO - A aroeira-do-sertão é uma espécie nativa do Brasil que está sendo explorada de forma predatória. Como a população desta espécie vem sendo reduzida, técnicas de preservação são fundamentais, como a conservação em nitrogênio líquido (NL). Objetivou-se avaliar diferentes soluções crioprotetoras na criopreservação em NL (-196 °C) de sementes de aroeira-do-sertão. Os tratamentos foram: controle (7,5±1,5 ° C); NL sem crioprotetor; sacarose 0,4 mol L^{-1}; sacarose 0,8 mol L^{-1}; glicerol 1 mol L^{-1}; glicerol 2 mol L^{-1}; PVS_1 (solução de vitrificação de plantas); PVS_2; PVS_2 + 1% floroglucinol e PVS_3. As sementes permaneceram congeladas por 120 dias. Antes da criopreservação, as sementes foram caracterizadas quanto à germinação e teor de água. Ao sétimo dia avaliaram-se diversos parâmetros de germinação e após 150 dias de cultivo, variáveis de crescimento das plantas. As sementes apresentaram 9,2% de teor de água e 74% de germinação antes da criopreservação. No controle, a germinação foi de 55%, enquanto na criopreservação, variou de 61 a 69%, mostrando efeito positivo da criopreservação, mesmo sem o uso de crioprotetores (69%). Observou-se modelo trifásico de absorção de água nas sementes, onde o NL promoveu antecipação da germinação, que se iniciou com 56 horas, enquanto o controle iniciou a germinação após 66 horas. As plantas após 150 dias de cultivo apresentaram desenvolvimento satisfatório de suas partes conforme verificado pelo índice de qualidade de Dickson (IQD). O uso de crioprotetores não influencia a germinação e crescimento inicial das plantas. As sementes de aroeira-do-sertão com 9,2% de teor de água podem ser criopreservadas em NL sem crioprotetores.

Palavras-chave: Conservação de sementes. Nitrogênio líquido. Espécie arbórea.

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INTRODUCTION

Aroeira-do-sertão (*Astronium urundeuva* M. Allemão Engl.; Anacardiaceae) has a high economic value due to the pharmaceutical properties of its leaves, barks, and roots, and due to its use for recovering degraded areas (SOUZA et al., 2020; SILVA et al., 2021). This species is heliophile and intermediately tolerant to low temperatures and presents slow growth. It is found mainly in the Northeast, but can also be found in the Southeast and Central-West regions of Brazil (SOUZA et al., 2020; SILVA et al., 2021).

Despite its wide dispersion in South America, the exploration of aroeira-do-sertão has compromising the conservation of populations in their habitats (CNCFLORA, 2022). The destruction and decrease of their habitats and the historical intensive exploration due to the wood quality of this species are among the main threats (LAVÔR; LAVOR; SANTOS, 2021). Thus, it is necessary to use technologies for the preservation of seeds of this plant species.

Cryopreservation is one of the technologies used to preserve plant species, which consists in maintaining the biological material conserved under low temperature for long periods of time, in general, using liquid nitrogen (-196 °C) (COELHO; GONÇALVES; ROMANO, 2020). Several plant materials can be cryopreserved; however, seeds have been used because they enable the maintenance of genetic variability.

However, seed preservation requires caution, considering that each species has specific characteristics related to storage conditions (BARROZO et al., 2022). Different results related to seed water content can be found at the end of maturation (CUNHA et al., 2019). Orthodox seeds, such as those of aroeira-do-sertão, have a fast decrease in water content (up to 5-7%) with no occurrence of structural damages, providing ideal conditions for storage for long times. However, cryopreservation may cause damages to tissues due to the formation of ice crystals inside the cells. Thus, the seed water content is important for the development of cryopreservation protocols (ARAÚJO et al., 2019).

The obtaining of a correct cryopreservation process requires the use of cryoprotectant substances, which are essential for the survival of the cryopreserved material. The use of these substances decreases the freezing temperature, minimizing and/ or avoiding formation of ice crystals and decreasing possible damages to cells during freezing and thawing processes (ARAÚJO et al., 2019).

Dimethyl sulfoxide, ethylene glycol, glycerol, and sucrose are among the most used cryoprotectants. Plant vitrification solutions (PVS) can also be used; they are composed of a mixture of cryoprotectants at different concentrations (PAULA et al., 2020). However, the cryoprotectant type, concentration, and time of exposure of the material can result in damages to cells caused by a high chemical toxicity. The association of cryoprotectants with the different types of materials to be conserved should also be considered (ARAÚJO et al., 2019; PAULA et al., 2020).

Thus, the objective of this work was to evaluate different cryoprotectant solutions for cryopreservation of aroeira-do-sertão seeds in liquid nitrogen.

MATERIAL AND METHODS

Plant material and seed lot characterization

The experiment was carried out at the Laboratory of Seed Analysis of the Department of Agronomy of the State University of Londrina, Londrina, Paraná, Brazil. Aroeira-do-sertão seeds at the physiological maturity stage were collected in four parent trees in October 2019 in Guanambi, Bahia, Brazil (14°18'11.60"S, 42°41'04.38"W, and altitude of 591 m). The seeds were, then, stored in Kraft® paper bags for 30 days under refrigeration (7.5±1.5 °C) and relative humidity of 26±7%.

A sample of seeds was taken before the experiment for determination of water content and for germination tests, following the methodology described in the Rules for Seed Analysis (BRASIL, 2009). The seed water contents were obtained using four replications of 2.0 g of seeds, which were placed in a forced air circulation oven at 105±3 °C for 24 hours. The results were expressed as percentages.

The germination test was carried out using four replications of 50 seeds, distributed in transparent acrylic boxes (11×11×3 cm) containing a blotter paper sheet moistened with distilled water at a rate equivalent to 2.5-fold the dry substrate weight. The boxes were, then, maintained in a germination chamber at 25 °C and photoperiod of 8 hours and evaluated for germination percentage on the seventh day.

Arrangement of treatments

The seeds were placed in 2.0 mL cryotubes; each container contained 50 seeds, totaling 200 seeds per treatment, as described in Table 1.

Table 1. Arrangement of treatments (T) with cryoprotectant solutions used for cryopreservation of seeds of aroeira-do-sertão (*Astronium urundeuva* M. Allemão Engl.).

T1	Without cryoprotectant solution or immersion in liquid nitrogen
T2	Without cryoprotectant
T3	Sucrose 0.4 mol L^{-1}
T4	Sucrose 0.8 mol L^{-1}
T5	Glycerol 1.0 mol L^{-1}
T6	Glycerol 2.0 mol L^{-1}
T7	Plant vitrification solution (PVS1)
T8	Plant vitrification solution (PVS2)
T9	Plant vitrification solution (PVS2) + 1% phloroglucinol
T10	Plant vitrification solution (PVS3)

Plant vitrification solutions (PVS) consist of compounds with different substances. The PVS_1 solution consisted of 19% glycerol (v v^{-1}), 13% ethylene glycol (v v^{-1}), 6% dimethyl sulfoxide (v v^{-1}), and sorbitol at 0.5 mol L^{-1} diluted in a MS medium (MURASHIGE; SKOOG, 1962; SAKAI; KOBAYASHI; OIYAMA, 1990) with half of the concentration of macronutrients. The PVS_2 solution consisted of 30% glycerol (v v^{-1}), 15% ethylene glycol (v v^{-1}), 15% dimethyl sulfoxide (v v^{-1}), and sucrose at 0.4 mol L^{-1} diluted in a MS medium with half of concentration of macronutrients (VENDRAME; FARIA, 2011). The PVS_3 solution consisted of 50% glycerol (v v^{-1}) and 50% sucrose (v v^{-1}) diluted in distilled water (TEIXEIRA et al., 2014).

The control treatment consisted of seeds stored in cryotubes at 8±2 °C. The treatment without cryoprotectant consisted of seeds directly immersed in liquid nitrogen (LN). The other treatments consisted of addition of 2.0 mL of the different cryoprotectant solutions in the cryotubes.

Seeds in the treatments with sucrose at 0.4 and 0.8 mol L^{-1} and glycerol at 1.0 and 2.0 mol L^{-1} remained exposed to the solutions for 20 minutes at room temperature (25±2 °C) and, then, immersed in LN. Seeds in the treatments containing PVS_1, PVS_2, PVS_2 + 1% phloroglucinol, and PVS_3 remained for 10 minutes in an ice bath (0 °C) and, then, they were immersed in LN. The cryotubes remained stored in a tank (45 L) containing LN (-196 °C) for 120 days; the volume was frequently monitored. The seeds were withdrawn and subjected to fast thawing in a water bath device (Evlab®, EV: 015, Brazil), with precision of 0.1 °C, at 40 °C for 90 seconds. The cryoprotectant solutions were removed from the cryotubes with the aid of a Pasteur pipette.

The seeds were sterilized in a 0.5% sodium hypochlorite solution for 5 minutes and, then, washed with distilled water before the germination tests; 2.0 mL of a 1.0 mol L^{-1} sucrose solution were added for 20 minutes and, then, 0.5 mL of this solution was withdrawn and the volume was completed with 0.1 mol L^{-1} sucrose solution for more 5 minutes. The seeds were then washed three times with distilled water and subjected to germination tests. A pre-test was carried out, focused on identifying the beginning and end of the germination.

The germination test consisted of germination of 50 seeds in blotter paper moistened with distilled water at the volume of 2.5 fold the paper dry weight; the seeds were placed in transparent acrylic boxes and taken to a germination chamber at 25 °C and photoperiod of 8 hours. The seeds were daily monitored for beginning of the germination process and, when at least one seed germinated, the third day was established as the first counting time; after stabilization of the number of germinated seeds, the test was ended, which occurred on the seventh day.

Imbibition curve

The data used to plot the imbibition curve were obtained from four replications of 25 seeds, totaling 100 seeds per treatment. The seeds were weighed with 1-hour intervals in the first 8 hours and, then, with 2-hour intervals for 72 hours. The seeds were arranged in blotter paper moistened with distilled water at the volume of 2.5 fold the paper dry weight and placed in transparent acrylic boxes (11×11×3 cm). The surfaces of the seeds were dried with the aid of germination paper before weighed; the weights (g) were obtained in an analytical balance (0.0001 g).

Seedlings evaluation (seven days)

The seedlings were evaluated through first germination counting, germination percentage, percentage of abnormal seedlings, germination speed index (GSI), mean time for germination (MTG), seedling length, and seedling dry weight.

The germination test was carried out as previously described; the first germination counting was carried out after three days, when the emission of the radicle of the first seedling occurred, and the result was expressed as percentage (BARROS et al., 2021). The number of normal and abnormal seedlings and non-germinated seeds were counted on the seventh day, as described in the Rules for Seed Analysis (BRASIL, 2009).

Simultaneously to the germination test, GSI was determined by daily counting of the number of germinated seeds until the seventh day, and

calculated through the formula described by Maguire (1962): GSI = E1/N1 + E2/N2 + ... + En/Nn, where E1, E2, and En are the number of normal seedlings in the first, second, and last counting; and N1, N2, and Nn are the number of days after sowing at the first, second, and last counting.

MTG was determined simultaneously to the germination test, as described by Barros et al. (2021); the number of germinated seeds was daily counted after the implementation of the test. This index represents the mean time required for germination, in which the weighting factor is the daily germination, calculated by the equation: $MTG = \frac{\text{G1T 1 +G2T 2 +..... GnTn}}{\text{G1 + G2.... + Gn}}$, where *MTG* is the mean time for germination (days required for reaching maximum germination) and G1, G2, and Gn are the number of germinated seeds in the times T1, T2, and Tn, respectively.

Ten seedlings of each replication were random selected in each treatment to determine total seedling length (cm), with the aid of a ruler; the results were expressed as the mean of each replication. The dry weight was evaluated by placing the seedlings in Kraft® paper bags and maintained them in a forced air circulation oven at 65 °C until constant weight. The seedlings were weighed (mg) in an analytical balance (0.0001 g).

Evaluation of seedlings (150 days)

The second part of the experiment was carried out after the end of the germination test; 20 seedlings of each treatment were acclimatized in a germination chamber and, then, transplanted into 415-mL plastic pots (7.5 cm height, 10.5 cm upper diameter, and 7.5 cm lower diameter) filled with a vermiculite substrate of medium granulometry.

The seedlings were maintained in a greenhouse under controlled environment (Van der Hoeven®) and covered with transparent polycarbonate plates and a diffuser, with a 50% shading screen (Aluminet®) under controlled temperature of 28±3 °C. Irrigation was carried out manually in the mornings, three times a week. After 150 days, the seedlings were evaluated for: shoot length and root length, with the aid of a ruler; shoot dry weight and root dry weight, obtained after drying in an oven and weighing; shoot dry weight to root dry weight ratio; and Dickson quality index (DQI). The stem base diameter was measured in the middle part of the stem base, using a caliper, to assess the DQI.

The DQI was obtained using the formula of Dickson, Leaf, and Hosner (1960):

$$\text{DQI} = \frac{TDW}{\frac{\text{SH}}{\text{SBD}} + \frac{\text{SDW}}{\text{RDW}}}$$

where *SH* is the shoot height (cm); SBD is the stem base diameter (mm); SDW is the shoot dry weight (g); RDW is the root dry weight (g); and TDW is the total dry weight (shoot + root) (g).

Experimental design and statistical analysis

A completely randomized experimental design was used. The statistical analysis for the germination test was carried out using 10 treatments with four replications of 50 seeds per transparent acrylic box, totaling 2.000 seeds. The analysis for the acclimatization of seedlings in the greenhouse were carried out considering the same 10 treatments with 20 replications per treatment, totaling 200 seedlings. The imbibition curves were obtained by fitting the data to cubic models in the R program.

The assumptions of normality of errors and homogeneity of variances were tested by the Shapiro -Wilk and Bartlett ($p \geq 0.05$) tests, respectively. The data were subjected to analysis of variance and Tukey's test ($p \leq 0.05$). The data were processed using the AgroReg package of the R program (R CORE TEAM, 2021; SHIMIZU; MARUBAYASHI; GONÇAVES, 2021).

RESULTS AND DISCUSSION

The aroeira-do-sertão seeds presented 9.2% water content and 74% germination before cryopreservation in LN. Several researches have shown that seed water content affects freezing processes. According to Paula et al. (2020), the recommended water content during freezing is below 10%, varying according to the species. Seeds of some species can be stored with water contents between 3% and 7% without affecting their physiological quality, as they have tolerance to dehydration and can maintain the vigor by decreasing their metabolic activity (PEREIRA et al., 2021a).

Regarding the changes in the aroeira-do-sertão seeds during the storage period, the germination percentage decreased 54% after three months of storage in refrigerator; under natural conditions, the germination was 36%. The seeds presented 85% germination and 8.0% water content at 5 days after the collection; however, the germination decreased to only 2% after seven months of storage (25±2 °C) (GUEDES et al., 2012).

Loss of viability and severe decrease in germination speed of aroeira-do-sertão seeds stored in a natural environment are little reported in the literature. A possible explanation is that the storage of this oilseed for long periods increases the chemical instability of fat acids, leading to lipid oxidation and deterioration of seeds, decreasing their physiological potential (GUEDES et al., 2012).

The low water content is one of the main

factors for the survival of seeds in cryogenic processes (FIOR; FIELDS; SCHWARZ, 2020). According to Bouteau (2022), a low seed water content can induce the quiescence state, i.e., a decrease in consumption of seed nutritive reserves when compared to a seed with higher water content, thus, maintaining their physiological quality and making viable the storage for long periods.

The results found for seeds of *Pyrostegia vesusta* (Ker Gawl.) Miers. after exposure to the LN confirm that the adoption of low water contents, between 4.4% and 6.5%, for long-term cryopreservation of this species is recommended (SALOMÃO; SANTOS; JOSÉ, 2020). Results found by Silva, Mata, and Duarte (2015) for *Punica granatun* L. seeds showed that water contents below 6% and above 12% decrease their germination and vigor when cryopreserved.

Regarding the phytometric analyses, germination percentage, germination speed index, seedling length, and seedling dry weight presented differences between treatments. However, first germination counting, abnormal seedlings, and mean time for germination were similar between treatments (Table 2).

Table 2. First germination counting (FGC), germination percentage (GP), abnormal seedlings (AS), germination speed index (GSI), and mean time for germination (MTG) of seeds, and seedling length (SL) and seedling dry weight (SDW) of aroeira-do-sertão (*Astronium urundeuva* M. Allemão Engl.) subjected to cryopreservation (-196 °C).

Treatment	FGC (%)	GP (%)	AS (%)	GSI	MTG (days)	SL (cm)	SDW (g)
Control*	3.0 a**	55.0 b	4.0 a	2.99 b	4.77 a	2.92 ab	0.104 b
Without cryoprotectant	8.0 a	69.0 a	4.0 a	4.25 a	4.15 a	3.88 a	0.129 a
Sucrose at 0.4 mol L^{-1}	3.0 a	64.0 ab	4.0 a	2.97 b	4.74 a	2.77 b	0.116 ab
Sucrose at 0.8 mol L^{-1}	5.0 a	64.0 ab	3.0 a	3.18 b	4.76 a	2.83 ab	0.114 ab
Glycerol at 1 mol L^{-1}	4.0 a	61.0 ab	3.0 a	3.21 ab	4.50 a	2.90 ab	0.117 ab
Glycerol at 2 mol L^{-1}	4.0 a	61.0 ab	3.0 a	3.01 b	4.89 a	2.95 ab	0.118 ab
PVS_1***	7.0 a	66.0 ab	3.0 a	3.40 ab	4.36 a	2.95 ab	0.115 ab
PVS_2***	7.0 a	65.0 ab	4.0 a	3.29 ab	4.48 a	3.12 ab	0.117 ab
PVS_2 + 1% F***	7.0 a	65.0 ab	3.0 a	3.23 b	4.35 a	2.97 ab	0.116 ab
PVS_3***	6.0 a	62.0 ab	3.0 a	3.47 ab	4.46 a	3.15 ab	0.120 ab
Coefficient of variation (%)	68.39	7.26	61.79	13.13	7.93	13.64	5.56

*Control = without cryopreservation, maintained at 10±2 °C.
**Means followed by the same letter in the columns are not different from each other by the Tukey's test ($p \leq 0.05$).
***PVS_1 = 19% glycerol (v v^{-1}), 13% ethylene glycol (v v^{-1}), 6% dimethyl sulfoxide (v v^{-1}), and sorbitol 0.5 mol L^{-1}, diluted in a MS medium; PVS_2 = 30% glycerol (v v^{-1}), 15% ethylene glycol (v v^{-1}), 15% dimethyl sulfoxide (v v^{-1}), and sucrose 0.4 mol L^{-1}, diluted in a MS medium; PVS_3 = 50% glycerol (v v^{-1}) and 50% sucrose (v v^{-1}) diluted in distilled water.

The treatment without cryoprotectants presented the highest germination percentage (69%), statically differing from the control (55%). No difference was found between the control and treatments with cryoprotectant solutions, presenting germination percentages varying from 55% to 66%. The germination speed indexes were different between treatments; the best results were found for the treatments without cryoprotectant, glycerol at 1 mol L^{-1}, PVS_1, PVS_2, and PVS_3, varying from 3.21 to 4.25; however, they did not significantly differ from the others.

The results for seedling length and seedling dry weight presented similar dynamics, with no differences between treatments using LN, with seedling lengths varying from 2.83 to 3.88 cm and seedling dry weights from 0.114 to 0.129 g. The seedling length found for the treatment without cryoprotectant (3.88 cm) differed only from the control (2.92 cm). The same occurred for seedling dry weight, with 0.104 g for the control and 0.129 g for the treatment without cryoprotectant.

No difference was found for first counting and mean time for germination, which varied from 3% to 8% and from 4.15 to 4.89 days, respectively. Regarding the abnormal seedlings, the treatments were not different from each other, varying from 3% to 4%.

Among the materials used for cryopreservation, seeds present advantages regarding survival and regeneration, as dehydration and freezing are faster and more uniform. In addition, it is a young material that presents small cells and a dense cytoplasm with few vacuoles, which means less free and available water for freezing, favoring the cryopreservation process and the maintenance of seed viability (OHSE, 2022).

The treatment without cryoprotectants was efficient for the maintenance of aroeira-do-sertão seeds after cryopreservation. Similar results were

reported by Araújo et al. (2019) for seeds of *Passiflora mucronata* Lam. treated with different cryoprotectants and cryopreserved in LN; the best results of germination (95%) were found in the treatment without use of cryoprotectants (direct immersion in LN).

Studies on cryopreserved seeds of *Sinningia leucotricha* (Hoehne) Moore. found higher results for germination when the seeds were immersed directly in LN (57%), whereas the treatments with different cryoprotectants resulted in germination percentages varying from 6% to 39% (STEGANI et al., 2017). *Pyrostegia venusta* (Ker Gawl.) seeds with water contents between 3.8% and 6.5% presented germinations between 89% and 98% after exposure to LN, which were higher than those obtained for non-frozen seeds (61% to 88%) (SALOMÃO; SANTOS; JOSÉ, 2020).

Aroeira-do-sertão seeds maintain their physiological quality when subjected to LN (-196 °C) because they are orthodox seeds. These seeds can be dehydrated to a low water content (3% to 7%) and present no damages caused by freezing or formation of ice crystals during the cryopreservation process; thus, they tolerate low water contents and normally start the germination process when hydrated under favorable conditions (BALLESTEROS; SLEZIAK; DAVIES, 2021).

One of the mechanisms of aroeira-do-sertão seeds for tolerance to dehydration and storage at low temperatures is related to the presence of lipids, sugars, and proteins, which perform functions connected to cell protection against biotic or abiotic stresses. Increases in these compounds ensure the maintenance of cell membrane integrity, cell differentiation, decrease in metabolism, efficiency of the antioxidant system, and presence of a repair system during rehydration (MATILLA, 2021).

The cryoprotectants maintained the viability and germination potential of the seeds; it can be explained by the use of cryoprotectants, which converted potentially freezable water into an amorphous non-crystalline solid of high viscosity, decreasing intracellular freezing, formation of ice crystals, and possible injuries to cells during the freezing and thawing phases (PEREIRA et al., 2021b).

Evaluations after cryopreservation are important to evaluate possible changes in morphogenetical responses (VENDRAME et al., 2014). However, the present study showed no negative responses to the treatments used (Figure 1).

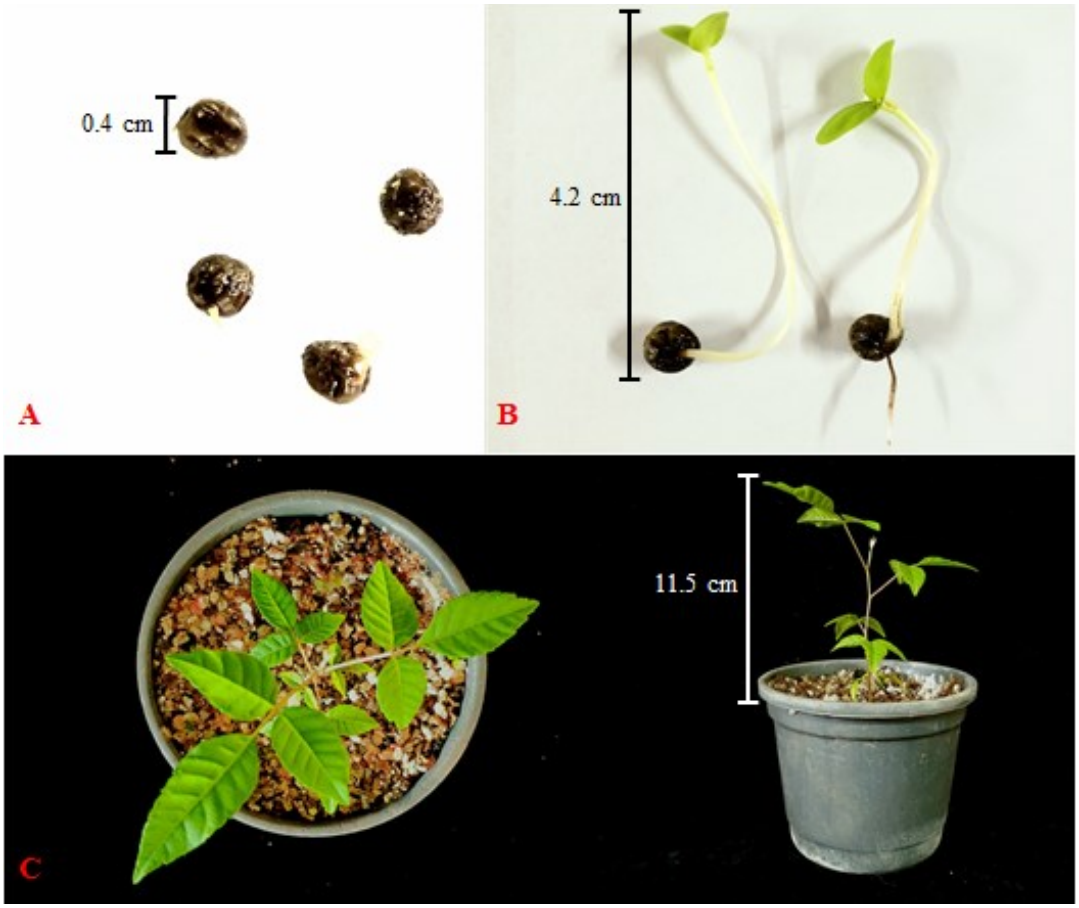

Figure 1. Detail of aroeira-do-sertão (*Astronium urundeuva* M. Allemão Engl.) seeds (A) and seedlings at 10 days (B) and 150 days (C) after germination, grown in a greenhouse after subjected to a cryopreservation process in liquid nitrogen (-196 °C).

The results showed no effects of addition of cryoprotectant substances after 150 days of growth in greenhouse, and no changes in root length (10.5 to 11.4 cm), shoot length (10.7 to 12.2 cm), root dry weight (0.015 to 0.019 g), shoot dry weight (0.069 to 0.075 g), and shoot dry weight to root dry weight ratio (3.85 to 4.31) (Table 3).

Table 3. Root length (RL), shoot length (SL), root dry weight (RDW), shoot dry weight (SDW), shoot dry weight to root dry weight ratio (SDW/RDW), and Dickson quality index (DQI) of aroeira-do-sertão (*Astronium urundeuva* M. Allemão Engl.) plants at 150 days after subjected to a cryopreservation process (-196 °C).

Treatment	RL (cm)	SL (cm)	RDW (g)	SDW (g)	SDW/RDW	DQI
Control*	11.2 a	11.8 a **	0.018 a	0.071 a	3.95 a	3.96 a
Without cryoprotectant	11.4 a	12.2 a	0.019 a	0.073 a	4.07 a	4.08 a
Sucrose at 0.4 mol L^{-1}	10.7 a	11.3 a	0.017 a	0.074 a	4.20 a	4.21 a
Sucrose at 0.8 mol L^{-1}	11.2 a	10.9 a	0.017 a	0.073 a	4.08 a	4.09 a
Glycerol at 1 mol L^{-1}	11.2 a	10.7 a	0.015 a	0.075 a	4.24 a	4.20 a
Glycerol at 2 mol L^{-1}	10.5 a	10.8 a	0.017 a	0.073 a	3.83 a	3.84 a
PVS_1***	10.7 a	10.8 a	0.016 a	0.069 a	4.10 a	4.11 a
PVS_2***	10.6 a	11.0 a	0.017 a	0.073 a	3.98 a	3.99 a
PVS_2 + 1% F***	10.9 a	11.2 a	0.018 a	0.075 a	4.24 a	4.21 a
PVS_3***	11.2 a	11.1 a	0.017 a	0.069 a	4.31 a	4.32 a
Coefficient of variation (%)	9.43	11.87	11.21	10.35	12.06	13.15

*Control = without cryopreservation, maintained at 10±2 °C.
**Means followed by the same letter in the columns are not different from each other by the Tukey's test ($p \leq 0.05$).
***PVS_1 = 19% glycerol (v v^{-1}), 13% ethylene glycol (v v^{-1}), 6% dimethyl sulfoxide (v v^{-1}), and sorbitol 0.5 mol L^{-1}, diluted in a MS medium; PVS_2 = 30% glycerol (v v^{-1}), 15% ethylene glycol (v v^{-1}), 15% dimethyl sulfoxide (v v^{-1}), and sucrose 0.4 mol L^{-1}, diluted in a MS medium; PVS_3 = 50% glycerol (v v^{-1}) and 50% sucrose (v v^{-1}) diluted in distilled water.

The Dickson quality index (DQI) showed no difference between treatments, varying from 3.84 to 4.32. DQI is used as a quality indicator of seedlings, as it determines the biomass robustness and balance, considering all parameters evaluated in the plant, such as height, root and shoot lengths, and stem diameter. Thus, the higher the DQI, the better the seedling quality (ABREU et al., 2019). The literature shows that there is no ideal DQI for the species studied. However, considering the DQI obtained and the lack of difference between treatments, the seedlings presented a satisfactory development after the cryogenic process.

The imbibition curve showed that the aroeira-do-sertão seeds presented a triphasic water absorption model at germination (Figure 2).

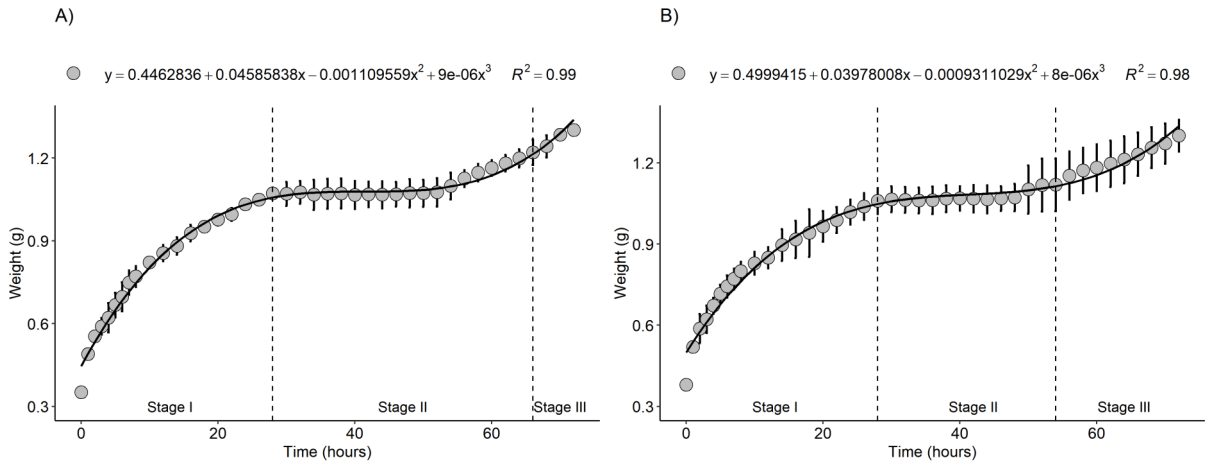

Figure 2. Water absorption curve of seeds of aroeira-do-sertão (*Astronium urundeuva* M. Allemão Engl.) stored at 10±2 °C (A) and at -196 °C (liquid nitrogen) (B).

The seeds absorb water rapidly at Stage I, termed imbibition (Figure 2). Seeds stored at 10±2 °C (control) presented an increase in seed weight from 0.35 to 1.07 g, i.e., a three-fold increase. The treatment with storage in LN resulted in seed weights from 0.38 to 1.05 g. Stage I lasted approximately 28 hours in both treatments. This stage establishes the beginning of transformation of simpler reserve substances to ensure the energy and nutrients needed for resuming the embryo growth (CAÇULA et al., 2022).

A stabilization in water absorption speed occurs in Stage II, and the metabolic processes of transcription and translation are resumed. This stage lasted 38 hours in the control and 28 hours in the LN treatment. This stage is characterized by expansion of the embryo and increases in production of enzymes, such as amylases, required for the metabolization of reserves and development of the embryo (RIBEIRO et al., 2021).

In Stage III, water absorption rate increases again due to the growth of the seedling, which begins to expand. It causes a reorganization of substances to form cytoplasm, protoplasm, and cell wall, resulting in the visible germination, when the protrusion of radicle occurs (NASCIMENTO; LOPES; ALEXANDRE, 2022). Seeds subjected to the LN treatment had faster germination, reaching this stage at 56 hours after imbibition, whereas the germination is the control started after 66 hours.

The imbibition curve showed that the germination started early for seeds exposed to LN. According to Salomão, Santos, and José (2020), exposure to LN can be used as a treatment to overcome dormancy in species with impermeable integument, and the immersion of seeds of some species into LN causes the formation of cracks in the integument, usually due to the pressure on them, which makes the seeds less rigid, facilitating the imbibition process and leading to an improvement in the germination process.

The results showed that the technique of cryopreservation in liquid nitrogen is viable and can be used for different types of cells, tissues, or organs of plants, resulting in maintenance of viability and genetic stability of the material. In addition, it is an easy technique that requires small spaces and has low cost (STEGANI et al., 2017; COELHO; GONÇALVES; ROMANO, 2020).

CONCLUSIONS

The use of cryoprotectants does not affect germination and initial growth of seedlings of aroeira -do-sertão. Seeds with 9.2% water content can be cryopreserved in liquid nitrogen with no need of cryoprotectants.

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