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I - Artigo Científico

Osmotic Regulators in the In Vitro Conservation of Cassava (*Manihot esculenta* Crantz)

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Abstract The use of osmotic regulators in *in vitro* germplasm conservation, aimed at reducing plant metabolism and growth, is one of the strategies employed for the proper management of these collections. This study aimed to evaluate the effect of different concentrations of mannitol and sorbitol, either isolated or in combination with sucrose, on the reduction of *in vitro* growth of cassava plants of the cultivar Aipim Brasil. For the experiments, shoot tips were cultivated in MS medium supplemented with 1.0 mg L^{-1} of thiamine, 100 mg L^{-1} of inositol, 0.02 mg L^{-1} of NAA, 0.04 mg L^{-1} of BAP, 0.05 mg L^{-1} of GA_3, 20 g L^{-1} of sucrose, and 2.4 g L^{-1} of Phytagel®, with the pH adjusted to 5.8. The culture was maintained in a growth room at 27 ± 1 ºC, with a photoperiod of 16 hours and a photon flux density of 22 µE m^{-2} s^{-1} for 30 days. For conservation, two experiments were conducted in a completely randomized design, in a factorial scheme (2 x 3), with two sucrose concentrations (0 and 20 g L^{-1}) and three concentrations of mannitol or sorbitol (0, 5, and 10 g L^{-1}), using the 8S medium as the base. Incubation was carried out at 21 ± 1 ºC, with a photon flux density of 18 µE m^{-2} s^{-1} and a 12-hour photoperiod. In both experiments, the absence of sucrose strongly impaired plant development. Mannitol showed a stronger growth-reducing effect compared to sorbitol. A concentration of 10 g L^{-1} of both sugars critically inhibited growth, demonstrating that the induced water stress was not tolerated by the plants, negatively impacting their development. The treatment with 20 g L^{-1} of sucrose, without the addition of mannitol or sorbitol, provided the best results under the conditions established in this study, enabling the conservation of plants for a period of 12 months.

Keywords: Euphorbiaceae; *In vitro* Conservation; Mannitol; Genetic Resources; Sucrose; Sorbitol.

(Reguladores osmóticos na conservação in vitro da mandioca (*Manihot esculenta* Crantz) A utilização de reguladores osmóticos na conservação de germoplasma *in vitro*, com o objetivo de reduzir o metabolismo da planta e seu crescimento, é uma das estratégias empregadas para o manejo adequado dessas coleções. O objetivo deste trabalho foi avaliar o efeito de diferentes concentrações de manitol e sorbitol, isolados ou em combinação com a sacarose, na redução do crescimento *in vitro* de plantas de mandioca da cultivar Aipim Brasil. Para a obtenção de plantas destinadas aos experimentos, ápices caulinares foram inoculados em meio MS, suplementado com 1,0 mg L^{-1} de tiamina, 100 mg L^{-1} de inositol, 0,02 mg L^{-1} de ANA, 0,04 mg L^{-1} de BAP, 0,05 mg L^{-1} de GA_3, 20 g L^{-1} de sacarose e 2,4 g L^{-1} de Phytagel®, com pH ajustado para 5,8. O cultivo foi realizado em sala de crescimento sob temperatura de 27 ± 1 ºC, fotoperíodo de 16 horas e densidade de fluxo de fótons de 22 µE m^{-2} s^{-1}, por 30 dias. Para a conservação, foram estabelecidos dois experimentos em delineamento inteiramente casualizado, em esquema fatorial (2 x 3), com duas concentrações de sacarose (0 e 20 g L^{-1}) e três concentrações de manitol ou sorbitol (0, 5 e 10 g L^{-1}), utilizando como meio básico o 8S. A incubação foi realizada a 21 ± 1 ºC, com intensidade de fluxo de fótons de 18 µE m^{-2} s^{-1} e fotoperíodo de 12 horas. Em ambos os experimentos, observou-se que a ausência de sacarose comprometeu fortemente o desenvolvimento das plantas. O manitol apresentou um efeito redutor de crescimento mais acentuado que o sorbitol. A concentração de 10 g L^{-1} de ambos os açúcares inibiu o crescimento de forma crítica, evidenciando que o estresse hídrico provocado não foi tolerado pelas plantas, interferindo negativamente no seu desenvolvimento. O tratamento com 20 g L^{-1} de sacarose, sem a adição de manitol ou sorbitol, promoveu o melhor resultado nas condições estabelecidas no trabalho, permitindo a conservação das plantas por um período de 12 meses.

Palavras-Chave: Euphorbiaceae; Conservação in vitro, Manitol; Recursos Genéticos; Sacarose; Sorbitol.

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Introduction

In recent years, the emergence of new technologies, land anthropization, and other factors have caused a rapid and profound erosion of genetic resources with real and potential value for food and agriculture. This erosion may lead to the extinction of invaluable agricultural materials, highlighting the critical role of germplasm collections in preserving these genetic resources (AMARAL et al., 2004; PRIYANKA et al., 2021; SALGOTRA; CHAUHAN, 2023; PINTO et al., 2024).

The strategies for germplasm conservation vary and depend on factors such as the reproductive system, sampling conditions, availability of physical, financial, and human resources, and political decisions (ENGELMANN, 2012; PRIYANKA et al., 2021). Effective *in situ* conservation requires fundamental ecological and genetic knowledge of populations. However, social, economic, and political factors in the targeted area may influence the success of this approach (ENGELMANN, 2012; PRIYANKA et al., 2021). On-farm conservation, performed in collaboration with family farmers, plays a significant complementary role to *in situ* conservation (CLEMENT et al., 2007; MAXTED et al., 2020; PUNEETH et al., 2024).

Seed conservation at low temperatures is ideal for orthodox species but not applicable to species with recalcitrant seeds or vegetative propagation (VITIS et al., 2020). Conversely, highly heterozygous species like cassava, which do not genotypically reproduce the mother plant, are conserved in field collections. While requiring minimal technology, these collections demand large experimental areas, labor, and are vulnerable to pests and diseases (FERREIRA et al., 2022; SAMPAIO FILHO et al., 2024).

These challenges underscore the need to develop alternative conservation techniques that can serve as security duplicates. *In vitro* conservation of micropropagated plants has been widely used for various economically important species, particularly those propagated asexually, and serves as an effective security duplicate (CANTO et al., 2004; SOUZA et al., 2009; CARVALHO et al., 2017; SOUZA et al., 2019; 2020; SILVA et al., 2021). A key advantage of this technique is the elimination of risks associated with field collections, along with the ability to maintain a large number of plants in a limited space (SILVA et al., 2016; 2021).

The production of plants for conservation involves *in vitro* multiplication of accessions, requiring adjustments to slow plant growth (SILVA et al., 2016; 2021). A drawback of this strategy is the need for periodic subculturing, which is labor-intensive and susceptible to somaclonal variation (SILVA et al., 2016; 2021). Factors such as temperature, light intensity, osmotic concentration, and plant growth regulators influence plant growth and, when properly controlled, help extend subculturing intervals (SILVA et al., 2020; 2021).

The strategy of maintaining plants in slow or minimal growth rates has been successfully applied, particularly for the conservation of meristematic apices of various species (SOUZA et al., 2019; 2020; SILVA et al., 2016; 2020; 2021; RAI et al., 2022). This technique drastically reduces plant metabolism without compromising viability by inducing osmotic stress, reducing light intensity or temperature, adding growth retardants, or decreasing the concentration of salts and organic components in the culture medium (SILVA et al., 2016; BENELLI et al., 2022).

Osmotic agents such as mannitol and sorbitol, when added to the culture medium, remove excess intracellular water through osmotic gradients, promoting slower growth (THORPE et al., 2008; BENELLI et al., 2022; OLIVEIRA; ALOUFA, 2022). Osmotic stress occurs when differences in molecular concentration between the external and internal cell environments lead to water movement by osmosis, altering intracellular conditions and affecting plant growth (MADAKADZE; SENARATINA, 2000; MOREIRA et al., 2012; BENELLI et al., 2022; OLIVEIRA; ALOUFA, 2022).

For greater efficiency in reducing cellular metabolism *in vitro*, it is recommended to combine factors such as incubation temperature, photon flux, photoperiod, and culture medium composition (BENELLI et al., 2022; OLIVEIRA; ALOUFA, 2022). Among medium components, sugars significantly influence plant growth. Mannitol and sorbitol, as alcoholized sugars with difficult metabolism, are more effective than sucrose in limiting growth. These sugars interact with sucrose content and conservation temperature, influencing plant metabolism (BENELLI et al., 2022; OLIVEIRA; ALOUFA, 2022).

Additionally, due to their highly hydroxylated nature, these sugars can replace water in cytoplasmic polysaccharides, maintaining normal enzyme and membrane functions under osmotic pressure (AFZAL et al., 2021; CARBÓ et al., 2023). Mannitol and sorbitol have been

evaluated as growth retardants in various species *in vitro*, including sugarcane (LEMOS et al., 2002), passion fruit (FARIA et al., 2006), bromeliads (MOREIRA et al., 2012), mangaba (JESUS et al., 2010; SANTOS et al., 2011), potato (GOPAL; CHAUHAN, 2010), *Poincianella pyramidalis* (SILVA et al., 2019), and *Epipactis flava* (LINJIKAO et al., 2024).

In cassava, studies conducted at CIAT (1984) evaluated the use of acetylsalicylic acid, silver nitrate, and different concentrations of mannitol or sorbitol, as well as their combinations, for germplasm maintenance. Results indicated that sucrose-sorbitol combinations could retard *in vitro* growth, although toxicity was observed in some cases (CIAT, 1984). Due to genotype dependency, further trials are necessary.

Unnikrishnan and Sheela (2000) evaluated sucrose and mannitol as osmotic agents in the *in vitro* conservation of six cassava cultivars, reporting significant effects. However, incubation temperatures of 27 ± 1 ºC led to rapid initial growth followed by quick deterioration, a condition undesirable for conservation. Some sucrose-mannitol combinations also showed deleterious effects.

The objective of this study was to evaluate the effects of osmotic regulators, such as mannitol and sorbitol, alone or combined with sucrose, on reducing cassava plant metabolism *in vitro* for germplasm conservation.

Materials and Methods

Plant Material

The cultivar Aipim Brasil (BGM-1660) was selected for this study due to its widespread use for various purposes and cultivation in different regions of Brazil.

Establishment of Explants

Cuttings from adult plants were planted in polyethylene pots containing a mixture of Plantmax® substrate and coconut fiber at a 2:1 ratio. After 21 days, apical regions of the shoots emerging from the cuttings, measuring 2 cm in length, were collected and placed in containers with distilled water. The disinfection process was performed under a laminar flow hood. Shoots were disinfected in 50% ethanol for three minutes, followed by treatment with 0.25% sodium hypochlorite for three minutes, and then rinsed three times in sterilized distilled water.

Following disinfection, shoot apices were excised under a stereomicroscope using a scalpel and forceps. The apices were inoculated into "4E" culture medium containing MS salts (MURASHIGE; SKOOG, 1962), supplemented with 1 mg L^{-1} thiamine, 100 mg L^{-1} inositol, 0.01 mg L^{-1} NAA (naphthaleneacetic acid), 0.04 mg L^{-1} BAP (benzylaminopurine), 0.05 mg L^{-1} GA_3 (gibberellic acid), 20 g L^{-1} sucrose, solidified with 2.4 g L^{-1} Phytagel®, and adjusted to a pH of 5.8.

Incubation was conducted in a growth chamber at a temperature of 27 ± 1 ºC, with a photoperiod of 16 hours and a photon flux density of 30 µmol m^{-2} s^{-1} for 30 days to establish the explants.

In vitro Multiplication of Plants

The multiplication stage consisted of three subcultures, using microcuttings approximately 1.2 cm long, each containing an apical bud and/or a lateral bud. The first subculture was conducted 60 days after the establishment phase, with subsequent subcultures performed at 60-day intervals.

In Vitro Conservation

Shoot apices obtained from the multiplication stage were inoculated into culture medium in test tubes (25 mm x 150 mm) sealed with PVC plastic and incubated in a conservation chamber. The incubation conditions were: temperature of 21 ± 1 ºC, photon flux density of 22 µmol m^{-2} s^{-1}, and a photoperiod of 12 hours.

The basal medium used was "8S," developed at the International Center for Tropical Agriculture (CIAT), Cali, Colombia (CIAT, 1984). This medium consists of MS salts and vitamins (MURASHIGE; SKOOG, 1962), supplemented with 0.01 mg L^{-1} NAA, 0.02 mg L^{-1} BAP, 0.1 mg L^{-1} GA_3, solidified with 2.4 g L^{-1} Phytagel®, and adjusted to a pH of 5.8.

Additionally, the medium was supplemented with sucrose, mannitol, and sorbitol in two separate experiments:

Experiment 1: Two sucrose concentrations (0 and 20 g L^{-1}) were tested in combination with three mannitol concentrations (0, 5, and 10 g L^{-1}).

Experiment 2: The same sucrose concentrations (0 and 20 g L^{-1}) were tested in combination with three sorbitol concentrations (0, 5, and 10 g L^{-1}).

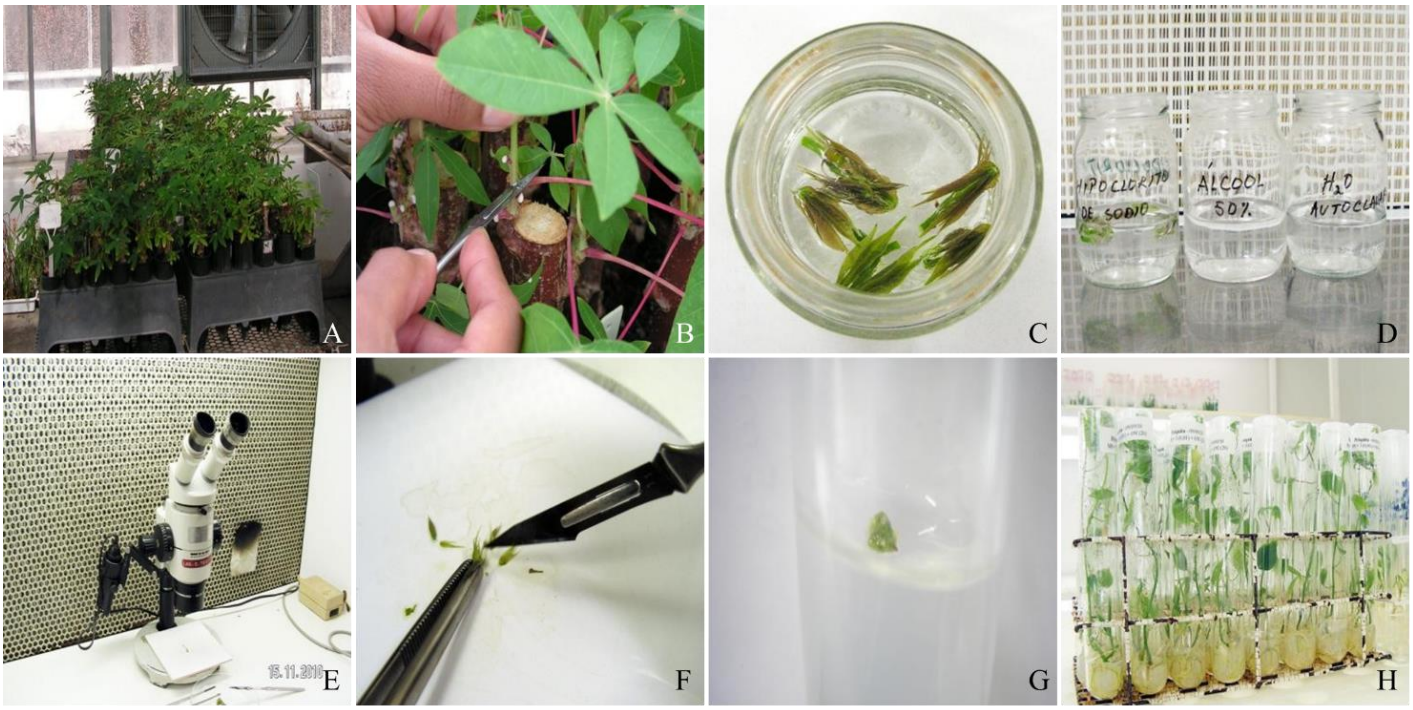

Figure 1. Steps for the establishment and multiplication of cassava (*Manihot esculenta* Crantz) for in vitro conservation studies. A) Plants cultivated in a greenhouse to provide starting material. B) Stem cuttings in pots in the greenhouse. C) Collection of shoot apices. D) Apices placed in distilled water. E) Disinfection using 50% ethanol, 0.25% sodium hypochlorite, and autoclaved water. F) Stereomicroscope used for shoot apex excision. G) Shoot apex excision with the aid of a scalpel and tweezers. H) Establishment of shoot apices in culture medium. I) Plant multiplication in culture medium.

Evaluations

Assessments were conducted after 12 months of conservation, considering the following variables: plant height (cm), number of microcuttings, number of green leaves, and number of senescent leaves.

Experimental Design

A completely randomized factorial design (2 x 3) was adopted, involving two sucrose concentrations and three mannitol or sorbitol concentrations, totaling six treatments with 20 replications. Each experimental unit consisted of one plant per test tube.

To homogenize data variance, statistical transformations were applied. Means were compared using Tukey's test at a 5% significance level, utilizing the statistical software SAS Institute (2022).

Results and Discussion

Experiment 1

The analysis of variance showed that both individual factors and their interaction significantly affected all analyzed variables. The absence of sucrose did not result in significant differences across all variables, regardless of the mannitol concentration used (Table 1 and Figure 2). In contrast, adding 20 g L^{-1} of sucrose produced positive results for all variables, underscoring its importance in plant development (Table 1 and Figure 2).

The best outcomes occurred in treatments containing 20 g L^{-1} of sucrose without mannitol, highlighting this treatment's superiority over sucrose absence. However, an exception was noted for the treatment with 10 g L^{-1} of mannitol, where sucrose levels did not produce significant differences (Table 1).

The growth-reducing effect was more pronounced in the presence of sucrose. Without this carbohydrate, mannitol doses did not produce statistical differences. However, with sucrose, higher mannitol concentrations intensified the growth reduction, likely due to osmotic stress caused by the combination of these sugars.

The results confirm mannitol's inhibitory effect on plant growth, independent of sucrose presence. This outcome can be explained by reduced water and nutrient absorption, caused by increased water potential in the culture medium, as reported by Benelli et al. (2022). Although mannitol is widely used for in vitro conservation due to its osmotic capacity, survival rates remain low for

several crops. For instance, in potatoes, only 37% of plants survived after nine months in 2 g L^{-1} mannitol at 27°C (FORTES; PEREIRA, 2001), rendering this treatment unfeasible.

Table 2. Plant height, number of microcuttings, number of green leaves, and number of senescent leaves in cassava plants (*Manihot esculenta* Crantz) as a function of different sucrose and mannitol concentrations.

Mannitol (g L^{-1})	Sucrose (g L^{-1})			
	0	20	0	20
	Plant height (cm)		Number of microcuttings	
0	2.26 aB	11.40 aA	1.35 aB	7.11 aA
5	1.94 aB	4.60 bA	1.16 aB	2.54 bA
10	1.49 aA	2.55 cA	1.00 aA	1.35 cA
CV (%)	19.42		18.49	
Means	4.07		2.44	
	Number of green leaves		Number of senescent leaves	
0	1.20 aB	4.55 aA	4.35 aB	6.72 aA
5	2.94 aB	3.63 bA	3.33 aB	5.45 aA
10	1.33 aA	1.64 cA	3.06 aA	3.41 bA
CV (%)	26.40		22.92	
Means	1.70		4.36	

Means followed by the same lowercase letter in columns and uppercase letter in rows do not differ significantly according to Tukey's test at a 5% probability level.

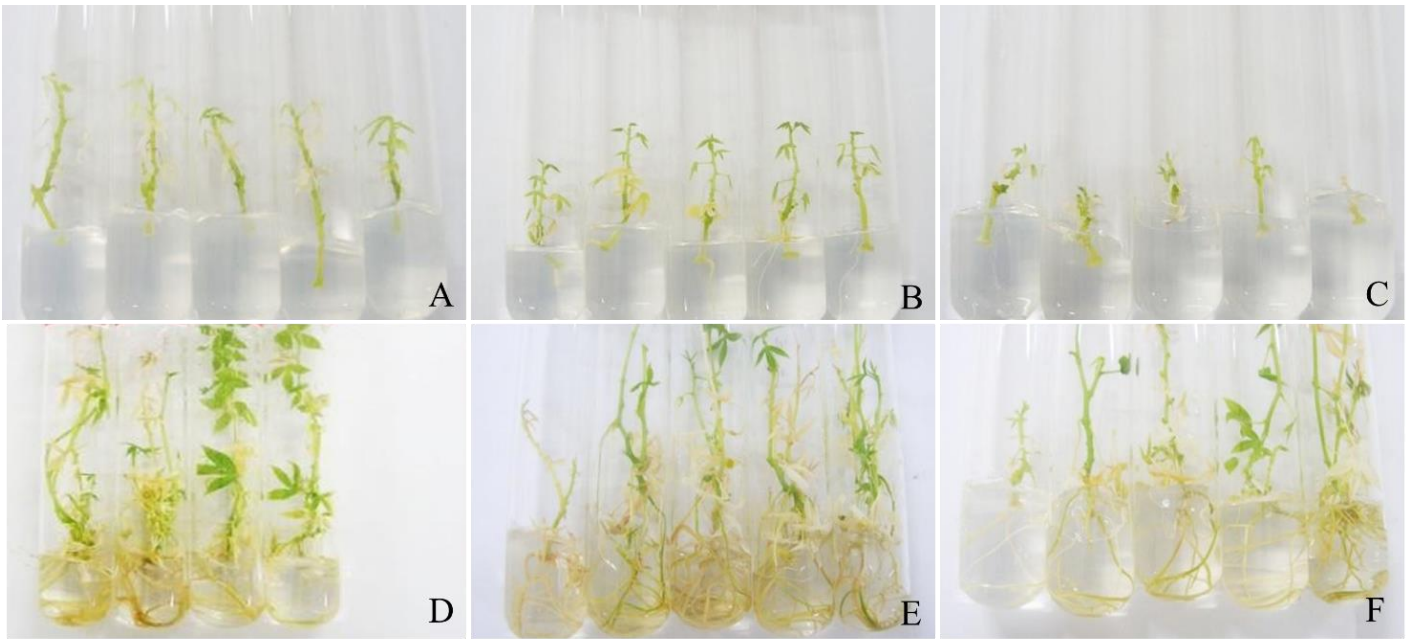

Figure 2. Cassava plants (*Manihot esculenta* Crantz) from different treatments after 12 months of in vitro conservation. A) 0 g L^{-1} sucrose + 0 g L^{-1} mannitol. B) 0 g L^{-1} sucrose + 5 g L^{-1} mannitol. C) 0 g L^{-1} sucrose + 10 g L^{-1} mannitol. D) 20 g L^{-1} sucrose + 0 g L^{-1} mannitol. E) 20 g L^{-1} sucrose + 5 g L^{-1} mannitol. F) 20 g L^{-1} sucrose + 10 g L^{-1} mannitol.

Sucrose's role as a carbon source was confirmed by the limited development observed in treatments without it. According to Grattapaglia and Machado (1998), Silva et al. (2016; 2020), and Souza et al. (2020), sucrose is the most commonly used carbon source in in vitro culture protocols, essential for cellular differentiation and proper plant development.

Other studies, such as those on bromeliads of the genus *Aechmea* Ruiz & Pav. (MOREIRA et al., 2012) and sugarcane (LEMOS et al., 2002), also evaluated combined sucrose and mannitol use, with varying results. However, elevated concentrations of this osmotic agent consistently showed toxicity.

In germinated *Hancornia speciosa* plants, Santos et al. (2011) reported that adding 15–20 g L^{-1} of mannitol to the culture medium allowed conservation for up to 180 days. However, the results of this experiment indicate that mannitol is unsuitable for the evaluated cassava variety, due to reduced numbers of green leaves and microcuttings, compromising regeneration and acclimatization after one year of cultivation.

Even at 5 g L^{-1}, mannitol presence did not prevent leaf senescence compared to treatments with sucrose. The highest mannitol concentration (10 g L^{-1}) resulted in less senescence but correlated with fewer microcuttings and green leaves. The lowest averages were recorded in medium containing 10 g L^{-1} mannitol and 20 g L^{-1} sucrose, confirming that high concentrations amplify its deleterious effects.

While mannitol is often used to simulate water deficit conditions, its application requires concentration adjustments to maintain plant regenerative capacity. Balancing these factors is essential to avoid excessive stress, which compromises the integrity of conserved material.

Root formation, observed only in treatments containing 20 g L^{-1} sucrose (Figure 2), was another relevant finding. Root development is critical for in vitro cassava conservation, as it reflects good plant development and facilitates direct acclimatization. Additionally, root presence enhances the success of conserved germplasm exchange.

Experiment 2

The analysis of variance revealed a significant interaction effect between sucrose and sorbitol for plant height (cm) and the number of microcuttings (Table 2 and Figure 3). However, this interaction did not significantly affect the number of green or senescent leaves.

The absence of sucrose did not result in significant differences in plant height or the number of microcuttings, regardless of sorbitol concentration. Conversely, treatments with 20 g L^{-1} sucrose significantly influenced these variables. Similar to the mannitol experiment, 5 g L^{-1} sorbitol produced results comparable to those with sucrose alone, without toxic or deleterious effects. However, absolute values remained lower than treatments with sucrose exclusively.

Root formation occurred in treatments containing 20 g L^{-1} sucrose in both experiments, whereas 10 g L^{-1} sorbitol was toxic and completely inhibited root system formation. Although the sucrose-sorbitol interaction did not significantly influence green or senescent leaf numbers, sucrose alone had a considerable impact. Its presence reduced the number of green leaves and increased senescent leaves, possibly due to the higher metabolism stimulated by the treatment, which promoted plant growth.

Overall, the absence of sucrose severely compromised plant development in both experiments, leading to low viability. Similar results were observed with high concentrations of osmotic agents, indicating that the induced water stress was intolerable for the plants, negatively affecting their growth and development.

Garzón (1987) reported different results when combining sucrose and mannitol for conserving six cassava cultivars. The combination was viable, but differences likely arose from significantly lower concentrations (10 g L^{-1} sucrose and 0.5 g L^{-1} mannitol).

The 20 g L^{-1} sucrose concentration yielded the best plant development in both experiments. In the mannitol experiment, even at low concentrations, plant metabolism decreased. However, this effect was not observed with sorbitol, highlighting differences in the action of these sugars.

The results indicate that for the evaluated cultivar, mannitol was less effective than sorbitol, as it reduced plant growth to the detriment of its propagation viability. Osmotic agents' efficiency in reducing cellular metabolism for in vitro conservation shows high variability depending on the species. Studies on *Annona muricata* L. (LEMOS; BAKER, 1998), sugarcane (LEMOS et al., 2002), potato (FORTES; PEREIRA, 2001), and *Podophyllum* L. (LATA et al., 2010) highlighted that reducing temperature was more effective than altering culture media, especially with mannitol and sorbitol.

Table 2. Plant height and number of microcuttings in cassava plants (*Manihot esculenta* Crantz) as a function of sucrose and sorbitol concentrations after 12 months of conservation.

Sorbitol (g L^{-1})	Sucrose (g L^{-1})			
	0	20	0	20
	Plant height (cm)		Number of microcuttings	
0	2.44 aB	8.94 aA	1.85 aB	6.00 aA
5	2.53 aB	6.81 aA	1.76 aB	4.50 aA
10	1.50 aA	2.82 bA	1.75 aA	1.00 bA
CV (%)	18.20		10.25	
Means	4.14		2.81	

Means followed by the same lowercase letter in columns and uppercase letter in rows do not differ significantly according to Tukey's test at a 5% probability level.

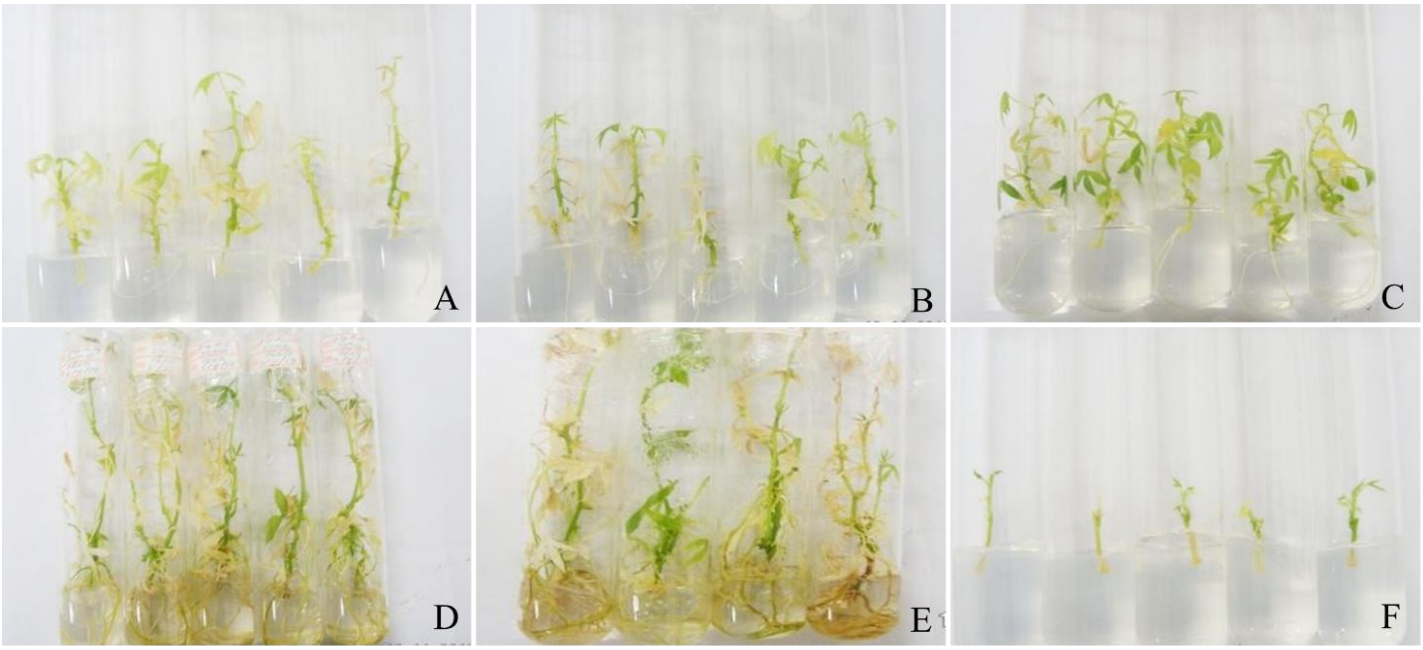

Figure 3. Cassava plants (*Manihot esculenta* Crantz) from different treatments after 12 months of in vitro conservation. **A)** 0 g L^{-1} sucrose + 0 g L^{-1} sorbitol. **B)** 0 g L^{-1} sucrose + 5 g L^{-1} sorbitol. **C)** 0 g L^{-1} sucrose + 10 g L^{-1} sorbitol. **D)** 20 g L^{-1} sucrose + 0 g L^{-1} sorbitol. **E)** 20 g L^{-1} sucrose + 5 g L^{-1} sorbitol. **F)** 20 g L^{-1} sucrose + 10 g L^{-1} sorbitol.

Positive sugar use outcomes were observed in bromeliads (MOREIRA et al., 2012), *Hancornia speciosa* (SANTOS et al., 2011), and *Passiflora* (FARIA et al., 2006). Nevertheless, osmotic agents continue to be investigated as a strategy for reducing cellular metabolism in in vitro culture, avoiding the use of plant growth regulators. According to Caldas and Buso (1998) and Lima et al. (2021), regulators directly affect plant metabolic pathways, potentially causing physiological or genetic disorders, while osmotic agents act by reducing the culture medium's water potential.

The results of this study suggest that the evaluated mannitol and sorbitol concentrations were too high, despite their recorded reducing effect. Trials with concentrations below 5 g L^{-1} are recommended to better evaluate these osmotic agents' viability for in vitro conservation.

Conclusion

The absence of sucrose hindered plant development in both experiments, underscoring its role as an essential carbon source for in vitro cultivation.

Mannitol or sorbitol in sucrose-free media did not significantly influence plant development, highlighting these agents' limitations without an available carbon source.

In media with 20 g L^{-1} sucrose, a synergistic effect between sucrose and osmotic agents (mannitol or sorbitol) reduced plant growth. This effect was proportional to the osmotic agents' concentration: higher concentrations led to greater growth reduction.

The 10 g L^{-1} concentration of both osmotic agents critically affected plant development, indicating unsuitability for in vitro conservation.

The treatment with 20 g L^{-1} sucrose alone provided the best results, ensuring adequate plant development and enabling conservation for 12 months.

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References

AFZAL, S.; CHAUDHARY, N.; SINGH, N.K. Role of soluble sugars in metabolism and sensing under abiotic stress. In: AFTAB, T., HAKEEM, K.R. **Plant Growth Regulators**. Cham. Springer, 2021, p. 305-334.

AMARAL, W.; THOMSON, L.; YAUNCHUR, A. Conservation of genetic resourcein the natural environment. In: FAO, FLD, IPGRI. **Forest genetic resource conservation and management:** overview, concepts and some systematic approach. Rome: International plant genetic resource Institut, v. 1, 2004, p. 1-4.

BENELLI, C.; TARRAF, W.; IZGU, T.; CARLO, A. In Vitro conservation through slow growth storage technique of fruit species: an overview of the last 10 years. **Plants**, v. 11, e. 3188, 2022.

CALDAS, L.S.; BUSO, J.A. **Cultura de tecidos e transformação genética de plantas**. Brasília: Embrapa. 1998. 132p.

CANTO, A.M.M.E.; SOUZA, F.V.D.; COSTA, M.A.P.C.; SOUZA, A.S.; LEDO, C. A.S.; CABRAL, J.R.S. Conservação *in vitro* de germoplasma de abacaxi tratado com paclobutrazol. **Pesquisa Agropecuária Brasileira**, v.39, p.717-720, 2004.

CARBÓ, R.; RODRÍGUEZ, E. Relevance of Sugar Transport across the Cell Membrane. **International Journal of Molecular Sciences**, v. 24, e. 6085, 2023.

CARVALHO, M.J.S.; OLIVEIRA, E.J.; SOUZA, A.S.; ALVES, L.B.; VENTURINI, M.T. Agronomic performance of cassava genotypes from the in vitro shoot tip culture submitted to clonal cleaning. **Bioscience Journal**, v. 33, p. 1144-1154, 2017.

CIAT. Centro Internaciona de Agricultura Tropical. **El cultivo de meristemas parala conservacion de germoplasma de yucca in vitro.** Cali, Colombia: CIAT, 1984, p. 40-44.

CLEMENT, R.C.; ROCHA, S.F.R.; COLE, D.M.; VIVAN, J,L. Conservação *on farm*. In: NASS, L.L. **Recursos Genéticos Vegetais**. Cap.15. Embrapa Recursos Genéticos e Biotecnologia, Brasília, 2007. p. 544.

ENGELMANN, F. Germplasm collection, storage, and conservation. In: ALTMAN, A.; HASEGAWA, P.M. **Plant Biotechnology and Agriculture**. Academic Press, 2012, p. 255-267.

FARIA, G.A.; COSTA. M.A.P.C.; JUNGHANS, T.G.; LEDO, C.A.S.; SOUZA, A.S. Efeito da sacarose e sorbitol na conservação invitro de *Passiflora giberti* N.E.Brown. **Revista Brasileira Fruticultura**, v.2, p.56-64, 2006.

FERREIRA, J.A.B.; SOUZA, F.V.D.; MOURA, H.C.P.; SOUZA, E.H.; CORTES, D.F.M.; CONCEICAO, J.Q.; LÊDO, C.A.S. Flowering map of papaya germplasm: support for genetic breeding and conservation programs. **Scientia Horticulturae**, v. 293, e. 110699, 2022.

FORTES, G.R.L.; PEREIRA, J.E.S. Preservação *in vitro* de batata com ácido acetilsalicílico e duas fontes de carboidratos. **Pesquisa Agropecuária Brasileira**, v. 36, p.1261-1264, 2001.

GARZÓN, D.I.A. **Efeito de la concentración de sacarose y manitol sobre el crescimiento *in vitro* de seis variedades de yuca, (*Manihot esculenta* Crantz.).** 1987. 140f. Dissertação (Universidade Del Valle), Departamento debiologia e química. Cali. Colômbia.

GOPAL, J.; CHAUHAN, N.S. Slow growth in vitro conservation of potato germplasm at low temperature. **Potato Research,** v. 53, p. 141–149, p. 2010.

GRATTAPAGLIA, D.; MACHADO, M.A. Micropropagação. In: TORRES, A.C.; CALDAS, L.S.; BUSO, J.A. **Cultura de tecidos e transformação genética de plantas**. Brasília: Embrapa-SPI: Embrapa-CNPH, v.1, 1998, p. 184-215.

JESUS, A.S.; LEDO, A.S.; LEDO, C.A.S. Conservação in vitro de mangabeira da região Nordeste do Brasil. **Ciência Rural**, v. 14, p. 8-12, 2010.

LATA, H. In vitro conservation of tropical plant germoplasm, a review. **Euphytica**, v. 57, p.227-243, 1991.

LEMOS, E.E.P.; BAKER, D. Shoot regeneration in response to carbon source on intermodal explants of *Annona muricata* L. **Plant Growth Regulation**, v. 25, p. 105-112, 1998.

LEMOS, E.E.P.; FERREIRA, M.S.; ALENCAR, L.M.C.; RAMALHO NETO, C.E.; ALBUQUERQUE, M.M. Conservação *in vitro* de germoplasma de cana-de-açúcar. **Pesquisa Agropecuária Brasileira,** v. 37, p. 1359-1364, 2002.

LIMA, A.P.P.S.; BASTOS, F.J.O; BRITO, A.L.; FERNANDES, G.B.; SANTANA, J.R.F. Modulation of culture medium on the ex situ conservation of *Neoregelia mucugensis* Leme (Bromeliaceae). Revista Caatinga, v. 34, p. 763-771, 2021.

LINJIKAO, J.; INTHIMA, P.; LIMMONGKON, A.; KONGBANGKERD, A. Mannitol and sorbitol concentration optimization for effective *Epipactis flava* Seidenf. *in vitro* slow growth storage. **In Vitro Cellular & Developmental Biology - Plant**, v. 60, p. 496-507, 2024.

MADAKADZE, R.M.; SENARATNA, T. Effect of grwth regulators on maturation of geranium (*Pelargonium* x *hortorum*) somatic embbryos. **Plant Growth Regulation**, v. 30, p. 34-45, 2000.

MAXTED, N.; HUNTER, D.; RÍOS R.O. On-Farm Conservation. In: MAXTED, N.; HUNTER, D.; RÍOS R.O. **Plant Genetic Conservation**. Cambridge University Press, 2020, cap. 9, p. 249-277.

MOREIRA, M.J.S.; BASTOS, M.J.S.M.; SOUZA, F.V.D.; ROCHA, M.A.C.; COSTA, M.A.P.C. Conservação in vitro de *Aechmea fasciata* (Lindley) Baker e *Aechmea miniata* Beer ex Baker (Bromeliaceae-Bromelioideae). **Magistra**, v. 24, p. 293-303, 2012.

MURASHIGE, T.; SKOOG, F. A revised médium for rapid growth and bioassays with tobacco tissue culture. **Physiology Plantarum**, v. 15, p. 473-497, 1962.

OLIVEIRA, K.S.; ALOUFA, M.A.I. Slow growth in vitro culture for conservation of *Hancornia speciosa* Gomes. **Floresta, v.** 52, p. 7– 16, 2022.

PINTO, A.V.; HANSSON, B.; PATRAMANIS, I.; MORALES, H.; OOSTERHOUT, C. The impact of habitat loss and population fragmentation on genomic erosion. **Conservation Genetics**, v. 25, p. 49–57, p. 2024.

PRIYANKA, V.; KUMAR, R.; DHALIWAL, I.; KAUSHIK, P. Germplasm Conservation: Instrumental in Agricultural Biodiversity-A Review. **Sustainability**, v. 13, e. 6743, 2021.

PUNEETH, G.M.; GOWTHAMI, R.; KATRAL, A.; LAXMISHA, K.M.; VASUDEVA, R.; SINGH, G.P.; ARCHAK, S. On-farm crop diversity, conservation, importance and value: a case study of landraces from Western Ghats of Karnataka, India. Scientific Reports, v. 14, e.10712, 2024.

RAI, M.K. Plant tissue culture targeting germplasm conservation. In Rai, A.C.; Kumar, A.; Modi, A.; Singh, M. **Advances in Plant Tissue Culture.** Academic Press, 2022, p. 205-221.

SALGOTRA, R.K.; CHAUHAN, B.S. Genetic Diversity, Conservation, and Utilization of Plant Genetic Resources. **Genes,** v. 14, e. 174, 2023.

SAMPAIO FILHO, J.S.; CAMPOS, M.S.; OLIVEIRA, E.J. Stability and genetic parameters for cassava yield attributes in the tropical humid region of Brazil. **Euphytica**, v. 220, e. 127, 2024.

SANTOS, M.C.; LEDO, A.S.; LEDO, C.A.S.; SOUZA, F.V.D.; SILVA JUNIOR, J.F. Efeito da sacarose e do sorbitol na conservação in vitro de segmentos nodais de mangabeira. **Revista Ciência Agronômica**, v. 42, p. 735-741, 2011.

SAS INSTITUTE. SAS/STAT. 15.2 User´s guide, Carry NC: SAS Institute, 2022.

SILVA, R.L.; FERREIRA, C.F.; LÊDO, C.A.S.; SOUZA, E.H.; COSTA, M.A.P.C.; SOUZA, F.V.D. Viability and genetic stability of pineapple germplasm after 10 years of in vitro conservation. **Plant Cell, Tissue and Organ Culture**, v.127, p. 123-133, 2016.

SILVA, R.L.; SOUZA, E.H.; PELACANI, C.R.; JESUS, J.S.; SOUZA, C.P.F.; SOUZA, A.B.P.; SOUZA, F.V.D. Validation of in vitro conservation of pineapple germplasm for ten years based on field morphological characterization. Genetic Resources and Crop Evolution, v. 68, p. 2051-2060, 2021.

SILVA, S.S.S.; SOUZA, E.H.; NEPOMUCENO, C.; SOUZA, F.V.D. ; COSTA, M.A.P.C. Micropropagation and in vitro conservation of *Alcantarea nahoumii* (Bromeliaceae), an endemic and endangered species of the Brazilian Atlantic Forest. **Acta Scientiarum. Biological Sciences**, v. 42, e. 52940, 2020.

SILVA, T.S.; NEPOMUCENO, C.F.; SOARES, T.L.; SANTANA, J.R.F. In vitro conservation of *Poincianella pyramidalis* (Tul.) L.P.Queiroz under minimal growth conditions. **Ciência e Agrotecnologia**, v. 43, e. 014519, 2019.

SOUZA, A. S.; JUNGHANS, T.G.; SOUZA, F.V.D.; SEREJO, J.A.S; NETO, H. P.; MENESES, M.C.; SILVEIRA, D.G.; SANTOS, V. da S. Micropropagação da mandioca. In: JUNGHANS, T.G.; SOUZA, A. S. **Aspetos Práticos da Micropropagação de Plantas**. Cruz das Almas: Embrapa Mandioca e Fruticultura Tropical, 2009, , p. 324-331.

SOUZA, F.V.D.; REBOUCAS, S.R.; SOUZA, E.H.; PEIXOTO, C.P. In vitro conservation of mango (*Mangifera indica* L.) Ubá and Carlota cvs. through culturing immature embryos. **Anais da Academia Brasileira de Ciências**, v. 92, e. 20190400-12, 2020.

SOUZA, F.V.D.; SANTOS, M.T.; SOUZA, E.H.; CARVALHO, A.C.P.P.; LEDO, C.A.S. In vitro nodal segment cultivation of wild accessions of pineapple. **Acta Horticulturae**, v. 1239, p. 129-136, 2019.

THORPE, T.; STASOLLA, C.; YEUNG, E.C. The components of plant Tissue Culture Media II: Organic additions and pH Effects, and Support Systems. In: GEORGE, E.F.; HALL, M.A.; DE KLERK, G.J. **Plant propagation by Tissue Culture**, v.1, 3rd ed, Netherlands: The Background, Springer, 2008, p.115-155.

UNNIKRISHNAN, M.; SHEELA, M.N. Studies on midia, explants and incubation conditions for in vitro conservation of cassava germoplasm. In: CARVALHO, L.J. C. B.; THRO, A.M.; VILARINHOS, A.D. **Cassava biotechnology**. Brasília. Embrapa Recursos genéticos e Biotecnologia. 2000, p.425-430.

VITIS, M.; HAY, F.R.; DICKIE, J.B.; TRIVEDI, C.; CHOI, J.; FIEGENER, R. Seed storage: maintaining seed viability and vigor for restoration use. **Restoration Ecology**, v. 28, p. 249–255, 2020.