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Effect of polyethylene glycol and silver nitrate on the *in vitro* preservation of *Manihot esculeta* Crantz

Efeito do polietilenoglicol e do nitrato de prata na conservação in vitro de Manihot esculeta Crantz

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ABSTRACT

Cassava (Manihot esculenta Crantz) plays a key role in Brazilian agriculture, but its genetic variability is under threat, requiring effective conservation strategies. This study aimed to evaluate the impact of polyethylene glycol (PEG) and silver nitrate ($AgNO_3$) on the *in vitro* conservation of the regional variety 'Amarelona', with a view to identifying concentrations that favor the maintenance of plant viability and controlled growth. Four concentrations of PEG (5, 10, 20, and 40 g/L) and two concentrations of $AgNO_3$ (5 and 10 mg/L) were applied, in addition to a control, in an experiment with seven treatments and 24 replicates, conducted for up to 180 days. The analyses included shoot height, number of leaves and shoots, root length, and fresh and dry mass of the shoot and root parts. The results showed that a concentration of 10 g/L PEG was the most effective for maintaining vegetative growth with long-term viability, while higher doses led to inhibition and senescence. $AgNO_3$, on the other hand, had negative effects in both concentrations, resulting in the death of plants throughout the experiment. It is concluded that PEG at 10 g/L is a promising solution for the *in vitro* conservation of cassava, ensuring the maintenance of viability, while the use of $AgNO_3$ at the concentrations tested severely compromised plant vitality.
KEYWORDS: Cassava. PEG. Silver nitrate.

RESUMO

A mandioca (Manihot esculenta Crantz) desempenha um papel fundamental na agricultura brasileira, mas a sua variabilidade genética está ameaçada, exigindo estratégias eficientes de conservação. Este estudo teve como objetivo avaliar o impacto do polietilenoglicol (PEG) e do nitrato de prata ($AgNO_3$) na conservação *in vitro* da variedade regional 'Amarelona', visando identificar concentrações que favoreçam a manutenção da viabilidade e crescimento controlado das plantas. Foram aplicadas quatro concentrações de PEG (5, 10, 20 e 40 g/L) e duas de $AgNO_3$ (5 e 10 mg/L), além de um controle, em um experimento com sete tratamentos e 24 repetições, conduzido por até 180 dias. As análises incluíram altura da parte aérea, número de folhas e brotos, comprimento da raiz e massa fresca e seca das partes aérea e radicular. Os resultados mostraram que a concentração de 10 g/L de PEG foi a mais eficiente para manter o crescimento vegetativo com viabilidade a longo prazo, enquanto doses mais elevadas levaram à inibição e senescência. Já o $AgNO_3$, em ambas as concentrações, apresentou efeitos negativos, resultando na morte das plantas ao longo do experimento. Conclui-se que o PEG a 10 g/L é uma solução promissora para a conservação *in vitro* da mandioca, garantindo a manutenção da viabilidade, enquanto o uso de $AgNO_3$ nas concentrações testadas comprometeu severamente a vitalidade das plantas.
PALAVRAS-CHAVE: Mandioca. PEG. Nitrato de prata.

INTRODUCTION

Manihot esculenta Cranz, native to South America, is known by the popular names: cassava, manioc, aipim, etc. Of the order Euphorbiales, family Euphorbiaceae, genus *Manihot*, a diversity of species is found in Brazil, but a significant decrease in its genetic variability has already been observed (ROCHA et al. 2020). In Brazil, cassava plays an important role in the economy for small and large producers, being of interest at the regional and industrial levels, with its production focused on products for human and animal consumption (RONKO et al. 2020). However, cassava productivity in certain regions of the Eastern Amazon is still low, compromising local agricultural potential. The main factor is related to crop diseases such as root rot, which pose threats such as the loss of valuable accessions among cassava varieties, affecting the genetic diversity and productive potential of the crop (ABRELL et al. 2022).

In vitro plant conservation has become an essential technique in germplasm preservation (CHANDRAN et al. 2023), especially for agriculturally important species such as cassava. This method offers an effective alternative to *ex situ* conservation, allowing genetic material to be maintained under controlled conditions, minimizing the space required and the costs associated with storage (RADOMIR et al. 2023). However, the success of *in vitro* conservation is directly linked to the careful selection of chemical agents and strict control of physical conditions in the environment, such as temperature and light. These factors play a decisive role in regulating plant growth, development, and viability (PIRES et al. 2022).

Among the agents, osmotic agents such as polyethylene glycol (PEG 6000 or 8000) stand out. These compounds are often incorporated into the growing medium to increase the osmotic concentration, simulating a drought environment. This increase in osmotic pressure causes a water deficit in plant cells, since their capacity to absorb water and nutrients is reduced, resulting in decreased growth of *in vitro* plants due to the reduced availability of water necessary for metabolic processes and cell growth (HERNÁNDEZ-PÉREZ et al. 2021, PRAMANIK et al. 2022).

Another agent is silver nitrate ($AgNO_3$), which is widely recognized for its ability to inhibit the production of ethylene, a plant hormone that regulates critical processes such as senescence and leaf abscission (DE OLIVEIRA FARIAS et al. 2020). Ethylene plays a key role in various stress responses and plant development, including fruit ripening, biotic and abiotic stress responses, and regulation of cellular aging (CHIEN & YOON 2024, HUANG et al. 2024). The application of $AgNO_3$ in *in vitro* cultivation can help prolong the viability of plant crops by reducing ethylene production, which in turn prevents premature senescence and leaf fall (MAHENDRAN et al. 2019).

Due to the great socioeconomic importance of cassava and the need to establish methodologies for the *in vitro* conservation of the species, the present study aimed to evaluate the impact of polyethylene glycol and silver nitrate on the i*n vitro* conservation of cassava, analyzing the effects of different concentrations of these agents on the vegetative growth and viability of the plants.

MATERIALS AND METHODS

The experiment was conducted at the *in vitro* Plant Micropropagation Laboratory, located at the Federal University of Western Pará. The regional 'Amarelona' variety

was used as plant material for this study. The matrices are part of the *in vitro* collection of the Maniva Tapajós Program.

Cassava seedling internodes, in the *in vitro* multiplication phase, were transplanted into MS medium (MURASHIGE & SKOOG 1962), supplemented with 0.01 mg/l ANA (naphthaleneacetic acid), 0.01 mg/l BAP (benzylaminopurine), and 0.01 mg/l AG3 (gibberellic acid). These internodes were carefully sectioned in a laminar flow cabinet, standardizing the cut to approximately 1 to 1.5 cm in length to be replanted in the experimental design.

The experiment was conducted using a completely randomized design (CRD), using different concentrations of polyethylene glycol (PEG) and silver nitrate ($AgNO_3$), in addition to a control with the standard multiplication medium mentioned above. The PEG concentrations were 5, 10, 20, and 40 g/L, while the $AgNO_3$ concentrations were 5 and 10 mg/L. Each treatment consisted of 24 replicates, totaling 24 test tubes per treatment. The culture media were prepared with 4.43 g/L of MS salts (MURASHIGE & SKOOG 1962), supplemented with 0.02 mg/L naphthaleneacetic acid (NAA), 0.04 mg/L 6-benzylaminopurine (BAP), and 0.05 mg/L gibberellic acid (GA_3), and solidified with 2 g/L Phytagel. In media containing PEG, sucrose was not added, while in media containing $AgNO_3$, sucrose was added at a concentration of 20 g/L. The pH of the media was adjusted to 5.7–5.8.

Each test tube, measuring 25 mm x 150 mm, received 10 mL of culture medium. The tubes were stored in a growth chamber at a controlled temperature of 27 °C ± 1 °C and a photoperiod of 16 hours of light. Treatment evaluations were performed at 60, 120, and 180 days after the start of the experiment.

The variables evaluated were: height of the aerial part (cm), number of shoots, number of living leaves, number of senescent leaves, root length (cm), fresh mass (mg), and dry mass (mg) of the roots and aerial part. The length of the aerial and root parts was measured using a caliper in millimeters (mm) and converted to centimeters (cm), while fresh and dry mass was determined using a precision scale. To obtain dry mass, a material drying oven with air recirculation was used at a temperature of 35 °C for 48 hours. For statistical analysis, the Dunnett test <0.05 was performed in SigmaPlot 14.0.

RESULTS

When comparing the results of treatments with polyethylene glycol (PEG) and silver nitrate ($AgNO_3$) across the three assessments, it was observed that both presented distinct responses. While treatment with PEG showed an increase in the evaluated means, silver nitrate showed a decrease in the evaluated criteria.

Regarding the height of the aerial part, the control group showed the greatest height in the three evaluations performed. The treatments with PEG and $AgNO_3$ showed different responses throughout the evaluations (Table 1). Among the treatments, the dosage of 10 g/l of PEG recorded the highest averages at the end of the experiment, with 0.985 cm, representing an 11-fold reduction compared to the control. On the other hand, the 20 g/l PEG dosage resulted in the lowest height values for the PEG treatments, with reductions of approximately 7x and 48x in the evaluations at 60 and 120 days, respectively, in addition to causing total plant mortality at 180 days.

The 10 mg/l dose of $AgNO_3$ showed the lowest values in the first assessment, with a height 9 times lower than that of the control, followed by the 5 mg/l dose, which showed height values 5 times and 60 times lower than the control at 60 and 120 days, respectively. At 180 days, treatment with $AgNO_3$ led to 100% mortality of the plants, making height measurements impossible.

Table 1. Evaluation of aerial part height growth (cm) over three evaluation periods: 60, 120, and 180 days, respectively.

	60 days	120 days	180 days
Control (Sucrose)	0.353	5.162	11.334
Polyethylene glycol 5 g/l	0.263	0.421*	0.434*
Polyethylene glycol 10 g/l	0.247	0.291*	0.985*
Polyethylene glycol 20 g/l	0.0509*	0.107*	0*
Polyethylene glycol 40 g/l	0.0805*	0.166*	0.227*
Silver nitrate 5 mg/l	0.115	0.0847*	0*
Silver nitrate 10 mg/l	0.038*	0*	0*

Averages marked with * in the columns differ significantly from each other according to the Dunnett test <0.05.

In terms of the number of leaves, the control group had the highest number of leaves in the three assessments carried out. The treatments with PEG and $AgNO_3$ showed different responses throughout the evaluations (Table 2). The dosage of 10 g/l of PEG recorded the highest averages of live leaves at the end of the experiment among the treatments, with an average of 2.8 leaves, representing a reduction of approximately 4x compared to the control. In contrast, the 20 g/l PEG dosage resulted in the lowest number of leaves for the PEG treatments, with reductions of approximately 5x and 3x at 60 and 120 days, respectively, in addition to causing total plant mortality at 180 days.

The 10 mg/L dose of $AgNO_3$ resulted in the lowest values at 60 days, with a height 20 times lower than that of the control. The 5 mg/L dose showed a number of leaves that was 2x and 4x lower than the control at 60 and 120 days, respectively. At 120 days, the 10 mg/L dose caused total plant mortality, while at 180 days, the 5 mg/L dose also led to 100% mortality. Treatment with $AgNO_3$ resulted in the complete death of the plants, making it impossible to count the number of leaves.

Table 2. Evaluation of the number of leaves at three evaluation periods: 60, 120, and 180 days, respectively.

	60 days	120 days	180 days
Control (Sucrose)	2.5	2.875	11.125
Polyethylene glycol 5 g/l	1.75	2.875	2,20*
Polyethylene glycol 10 g/l	2.375	2.5	2.816*
Polyethylene glycol 20 g/l	0,5*	0.875*	0*
Polyethylene glycol 40 g/l	0,25*	1	2.375*
Silver nitrate 5 mg/l	1.375	0.625*	0*
Silver nitrate 10 mg/l	0.125*	0*	0*

Averages marked with * in the columns differ significantly from each other according to the Dunnett test <0.05.

In the last assessment, the control group had the highest average number of senescent leaves. The PEG and $AgNO_3$ dosages showed distinct responses

throughout the evaluations (Table 3). At 60 days, both concentrations of 10 g/l and 20 g/l of PEG showed an average of 0.125 senescent leaves, a value higher than that of the control group. At 120 days, the responses of PEG concentrations diverged: the 10 g/L dose showed a 1x increase compared to the control, while the 20 g/L and 40 g/L doses showed reductions of 2x and 4x, respectively, compared to the control. At the end of the study, concentrations of 10 g/L and 20 g/L of PEG recorded the highest averages among the treatments, both with 3.625 senescent leaves, representing a 2x reduction compared to the control.

The dosage of 10 mg/L of $AgNO_3$ did not show leaf development in any of the evaluations. On the other hand, the concentration of 5 mg/L resulted, at 120 days, in an average of 0.625 senescent leaves, representing a twofold reduction in the number of senescent leaves compared to the control. However, at 180 days, this dose caused total inhibition of leaf senescence.

Table 3. Evaluation of the number of senescent leaves, under three evaluation periods: 60, 120, and 180 days, respectively.

	60 days	120 days	180 days
Control (Sucrose)	0	1.125	6.5
Polyethylene glycol 5 g/l	0	0.125	2*
Polyethylene glycol 10 g/l	0.125	1.5*	3.625*
Polyethylene glycol 20 g/l	0.125	0.5	3.625*
Polyethylene glycol 40 g/l	0	0.25	0.625*
Silver nitrate 5 mg/l	0.375	0.625	0*
Silver nitrate 10 mg/l	0	0*	0*

Averages marked with * in the columns differ significantly from each other according to the Dunnett test <0.05.

Regarding root length (cm), the control group showed the greatest development in all evaluations performed. While PEG dosages showed distinct responses over time, $AgNO_3$ concentrations resulted in total inhibition of root growth in all periods evaluated (Table 4). At 60 days, none of the treatments showed significant root growth. At 120 days, the 5 g/L PEG dosage recorded the highest average among the treatments, with an average root length of 0.084 cm, representing a 16-fold reduction compared to the control in the same period. At 180 days, the 40 g/L PEG dosage showed an average of 0.0434 cm, a 136-fold reduction compared to the control.

Table 4. Evaluation of root length (cm) over three evaluation periods: 60, 120, and 180 days, respectively.

	60 days	120 days	180 days
Control (Sucrose)	0.157	1.388	5.912
Polyethylene glycol 5 g/l	0	0.084*	0*
Polyethylene glycol 10 g/l	0	0*	0*
Polyethylene glycol 20 g/l	0	0.0211*	0*
Polyethylene glycol 40 g/l	0	0.04*	0.0434*
Silver nitrate 5 mg/l	0	0*	0*
Silver nitrate 10 mg/l	0	0*	0*

Averages marked with * in the columns differ significantly from each other according to the Dunnett test <0.05.

In terms of the number of shoots, the control group showed the highest shoot presence in the last two assessments. The PEG dosages showed distinct responses

throughout the evaluations (Table 5). At 60 days, doses of 10 g/L of PEG showed an average of 0.5 shoots, exceeding the control group's average of 0.357 during this period. At 160 days, the 5 g/L and 10 g/L PEG doses showed the highest averages at the end of the experiment, with 1.25 and 3.5 shoots, respectively, representing reductions of 8x and 3x compared to the control. In contrast, the dosage of 20 g/L of PEG resulted in total inhibition of shoot development, making counting impossible.

The $AgNO_3$ dosage of 10 mg/l did not show bud development in all evaluations, while the 5 mg/l dosage at 60 days resulted in the same average number of buds (0.345) as the control at 120 and 180 days, showing total inhibition.

Table 5. Evaluation of the number of shoots, under three evaluation periods: 60, 120, and 180 days, respectively.

	60 days	120 days	180 days
Control (Sucrose)	0.375	2.125	10.5
Polyethylene glycol 5 g/l	0.5	1	1.25*
Polyethylene glycol 10 g/l	0.375	1.25	3.5*
Polyethylene glycol 20 g/l	0	0*	0*
Polyethylene glycol 40 g/l	0	0.125*	0.5*
Silver nitrate 5 mg/l	0.375	0*	0*
Silver nitrate 10 mg/l	0	0*	0*

Averages marked with * in the columns differ significantly from each other according to the Dunnett test <0.05.

Regarding fresh shoot mass (mg), the control group showed the highest values in the last two evaluations (Table 6). The PEG and $AgNO_3$ dosages exhibited different responses throughout the experiment. At 60 days, the concentration of 5 g/L PEG showed an average of 5.063 mg of fresh mass, a value higher than that observed in the control group. At 120 days, this same dosage resulted in the highest fresh weight among the treatments, although with a 3-fold reduction compared to the control. At 160 days, the 10 g/L PEG dosage obtained the highest average fresh mass of the aerial part, with 14,275 mg, representing a 15-fold reduction compared to the control.

In the case of $AgNO_3$ dosages, at 60 days, the concentration of 10 mg/L presented a fresh mass of 0.175 mg, equivalent to a 22-fold reduction compared to the control, while the dosage of 5 mg/L registered a 6-fold reduction. At 120 days, the 10 mg/L dosage showed no results, and the 5 mg/L dosage exhibited a mass 68 times lower than the control. In the third evaluation, both $AgNO_3$ dosages resulted in 100% plant mortality, making it impossible to obtain fresh mass data.

Table 6. Evaluation of fresh shoot mass (mg) over three evaluation periods: 60, 120, and 180 days, respectively.

	60 days	120 days	180 days
Control (Sucrose)	3.862	28.413	219.125
Polyethylene glycol 5 g/l	5.063	7.888*	7.725*
Polyethylene glycol 10 g/l	4.175	5.837*	14.275*
Polyethylene glycol 20 g/l	0.613	1.113*	0*
Polyethylene glycol 40 g/l	0.362	2*	3.212*
Silver nitrate 5 mg/l	0.563	0.412*	0*
Silver nitrate 10 mg/l	0.175*	0*	0*

Averages marked with * in the columns differ significantly from each other according to the Dunnett test <0.05.

In relation to fresh root mass, the control group presented greater fresh root mass. PEG dosages exhibited distinct responses throughout the evaluations, while $AgNO_3$ concentrations demonstrated complete inhibition in all periods evaluated (Table 7). At 60 days, none of the treatments showed significant root growth; at 120 days, the 5 g/l and 20 g/l dosages showed averages of 0.213 and 0.1 mg, a decrease of 427x and 910x compared to the control, respectively. The dosage of 40 g/L of PEG obtained the highest average fresh root mass at the end of the experiment, with a weight of 0.05 mg, representing a 1000-fold reduction compared to the control.

Regarding the dry mass of the aerial part, the control group presented the highest values in the last two evaluations performed. The PEG and $AgNO_3$ dosages exhibited different responses throughout the experiment (Table 8). At 60 and 120 days, the 5 g/L PEG dosage showed averages of 4.675 mg and 7.175 mg of dry mass, respectively, with a 1x increase and a 3x reduction compared to the control. At 160 days, the 10 g/L PEG dosage obtained the highest average dry mass of the aerial part, with 12.225 mg, representing a 10-fold reduction compared to the control.

Table 7. Evaluation of fresh root mass (mg) over three evaluation periods: 60, 120, and 180 days, respectively.

	60 days	**120 days**	**180 days**
Control (Sucrose)	1.675	91.813	54.075
Polyethylene glycol 5 g/l	0	0.213*	0*
Polyethylene glycol 10 g/l	0	0*	0*
Polyethylene glycol 20 g/l	0	0.1*	0*
Polyethylene glycol 40 g/l	0	0.075*	0.05*
Silver nitrate 5 mg/l	0	0*	0*
Silver nitrate 10 mg/l	0	0*	0*

Averages marked with * in the columns differ significantly from each other according to the Dunnett test <0.05.

Table 8. Evaluation of the dry mass of the aerial part (mg) under three evaluation periods: 60, 120, and 180 days, respectively.

	60 days	**120 days**	**180 days**
Control (Sucrose)	3.337	24.95	128.475
Polyethylene glycol 5 g/l	4.675	7.175*	6.412*
Polyethylene glycol 10 g/l	3.65	5.15*	12.225*
Polyethylene glycol 20 g/l	0.463	0.987*	0*
Polyethylene glycol 40 g/l	0.212	2.675*	2.638*
Silver nitrate 5 mg/l	0.425	0.328*	0*
Silver nitrate 10 mg/l	0.0875*	0*	0*

Averages marked with * in the columns differ significantly from each other according to the Dunnett test <0.05.

On the other hand, the 10 mg/L $AgNO_3$ dosage showed a dry mass of 0.0875 mg at 60 days, representing a 38-fold reduction compared to the control. The 5 mg/L dosage recorded masses of 0.425 mg and 0.328 mg, corresponding to reductions of 7 times and 76 times compared to the control, at 60 and 120 days, respectively. At 120 days, the dosage of 10 mg/L showed no results. At 180 days, both $AgNO_3$ dosages led to 100% plant mortality, making weighing impossible.

In relation to dry mass of the root system, the control showed higher dry root mass in the last two evaluations performed. The PEG dosages exhibited distinct responses throughout the evaluations, while the $AgNO_3$ dosages demonstrated complete inhibition in all periods evaluated (Table 9). At 60 days, the 5 g/L PEG dosage achieved an average dry mass of 4,675 mg, representing a threefold increase compared to the control group average. At 120 and 160 days, this same dosage showed averages of 7.175 mg and 6.412 mg of dry root mass, corresponding to reductions of 11x and 7x in relation to the control, respectively.

Table 9. Evaluation of root dry weight (mg) over three evaluation periods: 60, 120, and 180 days, respectively.

	60 days	120 days	180 days
Control (Sucrose)	1.4	81	45.638
Polyethylene glycol 5 g/l	4.675	7.175*	6.412*
Polyethylene glycol 10 g/l	0	0	0
Polyethylene glycol 20 g/l	0	0.0875*	0*
Polyethylene glycol 40 g/l	0	0.0625	0.025
Silver nitrate 5 mg/l	0	0*	0*
Silver nitrate 10 mg/l	0	0*	0*

Averages marked with * in the columns differ significantly from each other according to the Dunnett test <0.05.

DISCUSSION

A comparative analysis between polyethylene glycol (PEG) and silver nitrate ($AgNO_3$) reveals fundamental differences in their modes of action and effects on plants. These substances, although used in different contexts for *in vitro* conservation, play distinct roles and elicit varied responses in plants.

Polyethylene glycol (PEG) is widely used as an osmotic agent in studies of plant conservation and growth *in vitro*, due to its ability to induce simulated water stress that can affect plant development. Studies such as those by MARTÍNEZ-SANTOS et al. (2021) and HERNÁNDEZ-PÉREZ et al. (2021) highlight that PEG is effective in controlling water availability by creating a growing environment that simulates drought conditions without causing severe damage to plants.

In the present study, a concentration of 10 g/l of PEG resulted in adequate vegetative growth, with plants exhibiting lower average heights compared to the control group. Specifically, there was a decrease observed in the height of the aerial and root parts, number of leaves, fresh and dry masses of both the aerial and root parts. These results suggest that PEG, at a moderate concentration such as 10 g/l, can create controlled water stress that reduces plant growth rates without inducing stress severe enough to significantly compromise their long-term viability. SAHAT et al. (2022) state that this approach allows for controlled development, which is crucial for germplasm conservation, as it keeps plants in a state of slow growth, which is desirable for prolonging their shelf life under conservation conditions.

On the other hand, higher concentrations of PEG, such as 20 g/l and 40 g/l, showed significant adverse effects. A drastic reduction or total inhibition was observed. This can be attributed to the fact that higher concentrations of PEG increase the osmotic pressure of the growth medium, making it a highly stressful environment for plants. JOLAYEMI & OPABODE (2018) demonstrated that cassava varieties can

respond in different ways to osmotic stress, demonstrating the plasticity of some cassava varieties to tolerate drought conditions, as observed in genotypes TMS 05/1654, TME 419, and TMS 92/0326. These genotypes had reduced morphological characteristics, which contributes to the observed results, showing that the variety responds differently to PEG dosages, creating a condition that inhibits water and nutrient absorption, leading to cell dehydration and, consequently, affecting plant growth.

The premature senescence observed at high doses of polyethylene glycol (PEG) triggered an adaptive mechanism in plants, whereby explants sacrifice less vital parts to preserve resources and ensure the survival of essential parts. DUBOIS & INZÉ (2020) highlight that this behavior is characteristic of plants under osmotic stress, requiring optimization of internal resources to survive in adverse conditions. In the present study, plants exposed to 20 g/L of PEG showed significant inhibition in parameters such as shoot height, number of leaves, root length, number of shoots, fresh and dry mass of the shoot and root.

Silver nitrate ($AgNO_3$) is known to inhibit the production of ethylene, a plant hormone that regulates processes such as senescence and leaf abscission, potentially prolonging the life of plants and inhibiting pathogens by blocking ethylene receptors in plant cells (MAHENDRAN et al. 2019). Although in appropriate concentrations it may be beneficial in the conservation of plant germplasm *in vitro*, slowing down metabolism and seedling development, this study demonstrated that the application of $AgNO_3$ to cassava had significant negative effects on growth parameters, probably due to toxicity exacerbated by high concentrations of the compound. Therefore, concentration and exposure time to $AgNO_3$ are critical factors in determining its beneficial or harmful effects.

During the experiment, it was observed that the application of $AgNO_3$ led to plant mortality from the second assessment onwards, as indicated by the data on shoot height (Table 1), number of living leaves (Table 2), number of senescent leaves (Table 3), fresh mass of the aerial part (Table 5), and dry mass of the aerial part (Table 7). In addition, parameters such as root length (Table 4), fresh root mass (Table 6), and dry root mass (Table 8) showed total inhibition from the first assessment. These findings suggest significant toxicity of $AgNO_3$, which drastically compromised the viability and development of cassava plants.

According to SIDDIQI & HUSEN (2022), higher concentrations of $AgNO_3$ can be highly toxic to plants. The oxidative stress resulting from the application of $AgNO_3$ leads to the production of reactive oxygen species (ROS), which can damage membrane lipids, proteins, and nucleic acids in plant cells. This cellular damage compromises the physiological and biochemical processes of plants, leading to a series of deleterious effects that include growth inhibition, severe stress, and eventually cell death. In the context of this study, the toxicity of $AgNO_3$ resulted in a drastic reduction in the growth parameters and viability of cassava plants, highlighting that the concentration and exposure time to $AgNO_3$ are critical factors that need to be carefully controlled to avoid severe negative effects.

Another crucial factor to consider is the genotype of the plants. Studies, such as CARVALHO et al. (2012), show that citrus varieties can have different responses in

morphological characteristics when subjected to the same concentration of $AgNO_3$. This suggests that the response to $AgNO_3$ exposure is genotype-dependent, implying that certain cassava varieties may be more susceptible to $AgNO_3$-induced toxicity than others. This genotypic factor is critical for interpreting the results, as the specific variety of cassava used in this study may have a particular sensitivity to $AgNO_3$ that contributes to the negative effects observed.

Studies on cassava, such as that by NEVES et al. (2021), also indicate that the response to $AgNO_3$ can vary significantly between different genotypes. For example, when using a dosage of 2.5 mg/l of $AgNO_3$ for 150 days, an effective reduction in height in cm was observed for three different cassava genotypes. These results highlight the importance of considering genotypic variability when evaluating the effects of $AgNO_3$ and suggest that selecting more tolerant varieties may be a strategy to minimize the toxic effects of $AgNO_3$ in future experiments.

Analysis of the data obtained in the experiment reinforces that, although silver nitrate may have a potential benefit in controlled concentrations, its applications should be used with extreme caution. Excessive exposure to or high concentrations of $AgNO_3$ can induce severe oxidative stress and compromise plant health, leading to negative effects that outweigh the potential benefits of its use in germplasm conservation.

CONCLUSION

This study demonstrated that polyethylene glycol (PEG) can act as a promising agent in the *in vitro* conservation of the regional variety 'Amarelona', especially at a concentration of 10 g/L, which favored vegetative growth and plant viability. In contrast, high concentrations of PEG (20 g/L and 40 g/L) and silver nitrate ($AgNO_3$) resulted in significant growth inhibition and increased senescence. The results obtained suggest further research to evaluate the conservation potential of PEG at different concentrations and under different in vitro culture conditions.

NOTES

AUTHORS' CONTRIBUTIONS

Conceptualization, methodology, and formal analysis: Bruno Gabriel da Silva Costa, Ellen Gabrielle Ileno de Sousa, and Diogo Guilherme Araújo Sá; research: Bruno Gabriel da Silva Costa; resources, data curation, and theoretical framework: Tulio Silva Lara and Eliandra de Freitas Sia; writing – preparation of the original draft: Bruno Gabriel; writing – revision and editing: Ellen Gabrielle Ileno de Sousa and Diogo Guilherme Araújo Sá; visualization: Bruno Gabriel; supervision, project management, and funding acquisition: Tulio Silva Lara and Eliandra de Freitas Sia. All authors have read and agreed to the published version of the manuscript.

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STATEMENT BY THE INSTITUTIONAL REVIEW BOARD

Not applicable to studies that do not involve humans or animals.

INFORMED CONSENT STATEMENT

Not applicable because this study did not involve humans.

DATA AVAILABILITY STATEMENT

Data can be made available upon request.

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CONFLICTS OF INTEREST

There was no conflict of interest on the part of the authors.

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