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IN VITRO CULTURE OF ZYGOTIC EMBRYOS OF *Syagrus romanzoffiana* (CHAM.) GLASSMAN (ARECACEAE)

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Resumo

Cultivo in vitro de embriões zigóticos de Syagrus romanzoffiana (Cham.) Glassman (Arecaceae). *Syagrus romanzoffiana* (Cham.) Glassman (Arecaceae) popularmente conhecida como jerivá, é uma espécie de palmeira de ampla distribuição no Brasil. O jerivá está presente na alimentação de animais e do ser humano que consomem tanto o fruto quanto o palmito-jerivá. A espécie também apresenta importância medicinal, ornamental e ecológica. O cultivo *in vitro* facilita a germinação de *S. romanzoffiana*, pelo qual é possível obter uma alta resposta morfogenética. Desse modo, os objetivos do presente trabalho foram estabelecer um protocolo para a germinação *in vitro* de *S. romanzoffiana* a partir da cultura de embriões zigóticos e descrever uma metodologia para a avaliação da viabilidade dos embriões com uso de tetrazólio. Para isso, foram coletados frutos de *S. romanzoffiana*, realizada a excisão dos embriões, estes foram cultivados com ácido diclorofenoxiacético (2,4-D) ou ácido giberélico (GA_3), perfazendo dois experimentos. Além disso foram avaliados tempo de exposição e concentração do tetrazólio para a avaliação da viabilidade dos embriões. Os resultados evidenciaram que 39,4% dos embriões do cultivo com 2,4-D e 25% do cultivo com GA_3 se desenvolveram formando plântulas. Não foi encontrada diferença significativa de massa para as plântulas desenvolvidas com os tratamento 2,4-D e GA_3. No teste de tetrazólio 75% dos embriões de *S. romanzoffiana* se mostraram viáveis. O protocolo de germinação *in vitro* para *S. romanzoffiana* apresentou germinação (32,2%) com apenas 27% de contaminação dos meios de cultura.
Palavras-chave: embrião vegetal, palmeira, micropropagação, ácido diclorofenoxiacético (2,4-D), ácido giberélico (GA_3), tetrazólio.

Abstract

Syagrus romanzoffiana (Cham.) Glassman (Arecaceae) also known as queen palm, is a species of palm, widely distributed in Brazil. The queen palm is present in the food of animals and humans who consume both the fruit and the heart of palm. The species also has medicinal, ornamental and ecological importance. In vitro cultivation facilitates the germination of *S. romanzoffiana*, in which it is possible to obtain a high morphogenetic response. Thus, the objectives of the present work were to establish a protocol for the in vitro germination of *S. romanzoffiana* from the culture of zygotic embryos and to describe a methodology for the evaluation of the embryo viability with the use of tetrazolium. For this, fruits of *S. romanzoffiana* were collected, the embryos were excised and cultivated with dichlorophenoxyacetic acid (2,4-D) or gibberellic acid (GA_3), in two experiments. In addition, the exposure time and concentration of tetrazolium were evaluated for embryo viability. The results showed that 39.4% of the embryos cultivated with 2,4-D and 25% cultivated with GA_3 developed into seedlings. No significant difference in mass was found for seedlings developed with treatment with 2,4-D and GA_3. In the tetrazolium test, 75% of the *S. romanzoffiana* embryos were considered viable. The in vitro germination protocol for *S. romanzoffiana* showed satisfactory germination results (32.2%) with only 27% contamination of the culture media.
Keywords: plant embryo, palm tree, micropropagation, dichlorophenoxyacetic acid (2,4-D), gibberellic acid (GA_3), tetrazolium.

INTRODUCTION

Palm trees (Arecaceae) can be found around the world in the tropical and subtropical regions, they vary greatly in terms of species richness and morphology. There are around 2600 species and 181 genera in this family. Numerous species are important especially in rural communities as raw material for construction, fabrics, fuel, food, in addition to having medicinal and ornamental uses in large urban areas (DEL POZO *et al*., 2020; DE SOUZA *et al*., 2020). *Syagrus* Mart. is a genus of palm trees belonging to the tribe Cocoseae (subfamily Arecoideae), subtribe Attaleinae. Currently there are 69 species, 1 subspecies and 14 natural hybrids (NOBLICK, 2017; 2018; SOARES & GUIMARÃES, 2019; SANT'ANNA-SANTOS *et al*., 2023). In Brazil, *Syagrus* species occupy a variety of habitats, such as humid tropical forests, the Amazon and the Atlantic Forest, and also drier regions such as the Caatinga, Cerrado and rupestrian fields (NOBLICK, 2017).

Syagrus romanzoffiana (Cham.) Glassman popularly known as jerivá, is a palm tree native from South America distributed in Brazil, Paraguay, Argentina and Uruguay. Its fruit is important in feeding several species of animals. The fruit and the jerivá palm heart are edible for humans, in addition to having medicinal and ornamental importance for both urban landscaping and the ecosystem (NOBLICK, 2017). *S. romanzoffiana* has mainly a southern distribution, being found in different habitats in the semi-deciduous forests of the Paraná Basin and the Atlantic Forest, also in the steppes, restingas, fields and in the Araucaria forest (JORGE *et al*., 2021; NOBLICK, 2017; SOARES, 2023).

S. romanzoffiana seeds present a low germination rate (26%) as well as a low emergence speed (10%) due to the existence of a dormancy mechanism, evidenced by phenolic compounds that inhibit germination (OLIVEIRA *et al*., 2015). The in vitro cultivation is a tool that supports several lines of research in the areas of genetics, plant physiology, phytopathology, phytotechnology, and others. One of the uses for in vitro cultivation from the embryo is to enable the propagation of species whose seed dormancy may be difficult to overcome (BARRUETO CID; TEIXEIRA, 2015; NETO *et al*., 2020; RIBEIRO *et al*., 2011). There are few works concerning in vitro germination of *S. romanzoffiana*. Oliveira *et al*. (2015) carried out a germination experiment by burying *S. romanzoffiana* seeds and monitoring their development every month for a year. The maximum germination obtained was 26%, they also performed a tetrazolium test that showed 80% of seed viability.

The dichlorophenoxyacetic acid (2,4-D) is a synthetic auxin derived from the phenoxyacetic acid and also an herbicide widely used worldwide. As an auxin, 2,4-D can be used in non-toxic concentrations in order to mimic the effects of natural auxins and stimulate plant growth (FRÖHLICH *et al*., 2021). The use of 2,4-D to induce the growth of somatic embryos is described in the work of Halperin and Wetherell (1964). They showed that a callus was produced from any part of a carrot and when transferred to culture media with reduced levels of auxin these calluses formed somatic embryos. In other works, Tofanelli *et al*. (2014) used 2,4-D as an alternative auxin to indolebutyric acid (IBA) in rooting grapevine rootstock cuttings. Fröhlich *et al*. (2021) used 2,4-D to promote the development of soybean plants.

Synthetic gibberellic acid (GA_3) can be used to induce a plant growth and development response (PEDÓ *et al*., 2018). Rego *et al*. (2018) observed that gibberellic acid also acted in overcoming seed dormancy. Dias *et al*. (2013) used gibberellic acid to study its effects on the germination of *Butia capitata* (Arecaceae) seeds. In this work, *B. capitata* is classified as a species with shallow dormancy due to the efficiency of GA_3 in stimulating germination.

The tetrazolium test is based on dehydrogenase enzymes activity involved in the process of cellular respiration. These enzymes cause a tetrazolium salt reduction reaction, which only occurs in living cells and results in the formation of a compound with an intense red color in the tissues of the seed embryo, while non-viable tissues maintain their original color (FRANÇA-NETO; KRZYZANOWSKI, 2018; SOUSA *et al*., 2017; IOSSI *et al*., 2016). For each species, due to particular differences in the seed or in the embryo, it is necessary to develop an efficient and adapted tetrazolium test methodology. Other factors such as seed storage conditions, pre-conditioning can also change the evaluation of the test for different species (IOSSI *et al*., 2016).

We used the in vitro propagation methodology to evaluate the germination of *S. romanzoffiana* embryos in culture medium, containing two compounds that act as germination inducers. Dichlorophenoxyacetic acid (2,4-D) and gibberellic acid (GA_3) were to stimulate plant growth and to overcome seed dormancy. We collected the data about the development of embryos into seedlings and seedlings mass after the experiment ended. The tetrazolium test was also conducted for the embryos.

Thus, the objectives of the present work were to establish a protocol for the in vitro germination of *S. romanzoffiana* from the culture of zygotic embryos and to describe a methodology using tetrazolium to evaluate the viability of the embryos.

MATERIAL AND METHODS

Plant material

The fruits of *Syagrus romanzoffiana* (Cham.) Glassman were collected from parent plants in the urban region of Curitiba - PR in the months of October and November 2022. The parent plants are located near the Polytechnic *campus* of the Federal University of Paraná (UFPR) (location 1: lat: -25.449390, long: -49.233082; location 2: lat: -25.446515, long: -49.238470; location 3: lat: -25.439312, long: -49.238191). The fruits were stored under refrigeration (4ºC), in the Laboratory of Forestry Biotechnology, Department of Forestry Sciences at UFPR. A bench vise was used to break the seeds and access the embryo without injuries. The seeds were broken right before the inoculation of the embryos in the culture medium to reduce the chances of contamination, since the embryos were not disinfected during the experiments. All described experiments were carried out at the BiotecFlor.

Experiment with dichlorophenoxyacetic acid (2,4-D) and gibberellic acid (GA_3)

In both experiments with dichlorophenoxyacetic acid (2,4-D) and gibberellic acid (GA_3) treatments, embryos were inoculated in MS plant growth medium (MURASHIGE & SKOOG, 1962) with the addition of sucrose (30 g L^{-1}), agar (7 g L^{-1}) and 0.25% activated charcoal (MINARDI *et al*., 2011). The pH of the culture medium was adjusted to 5.8 and sterilized. Test tubes containing 10 mL of the culture medium were sealed with a plastic lid and completely wrapped with aluminium foil to avoid exposure to light. The tubes were kept with no light in a growth room at 25 ± 2°C for 21 days and then exposed to the light until the end of the experiment. The development of the embryos was evaluated for eight weeks, totalling 56 days since the inoculation of the embryos.

The treatment with dichlorophenoxyacetic acid (2,4-D) concentrations were: 0.00; 0.25; 0.50 and 1.00 mg L-1; and with gibberellic acid (GA_3): 0.0; 1.0; 2.0 and 4.0 mg L-1. The experimental design was completely randomized, with four replications and ten embryos inoculated per plot (4x4x10). After eight weeks in the growth room, the embryos were evaluated considering the ones that developed and formed seedlings, that did not develop into seedlings and those that showed contamination in the growth medium. The mass (g) of the embryos that developed and formed seedlings was quantified for each treatment and the data was subjected to analysis of variance (ANOVA) using the Sisvar software (FERREIRA, 2019).

Experiment with tetrazolium

To evaluate the embryos viability, we used the tetrazolium test. The embryos were subjected to three different tetrazolium concentrations (0.25%, 0.5% and 1%), in two imbibition times (three and five hours) and with four replications and ten explants per plot (3x4x10). The experiment was conducted at room temperature of 25 ± 2°C, and in the absence of light. After the exposure to tetrazolium, the embryos had their color analysed for each concentration and imbibition time differentiating those that acquired a reddish color as the viable ones, from the non-viable, that remained with the same color (BRASIL, 2009).

RESULTS

In the analysis of experiments with dichlorophenoxyacetic acid (2,4-D) and gibberellic acid, a qualitative classification of the final stage of embryo development was carried out (Figure 1). The three final stages of embryo into seedlings development were: 1. developed seedlings, featured with regions such as the eophyll and plumule (chlorophyll tissue) (Figure 1A, 1B e 1C); 2. dead seedlings, that experienced a period of development and died, with no sign of chlorophyll tissue and with the presence of necrosis (Figure 1D e 1E); and 3. the non-developed embryos (Figure 1F e 1G).

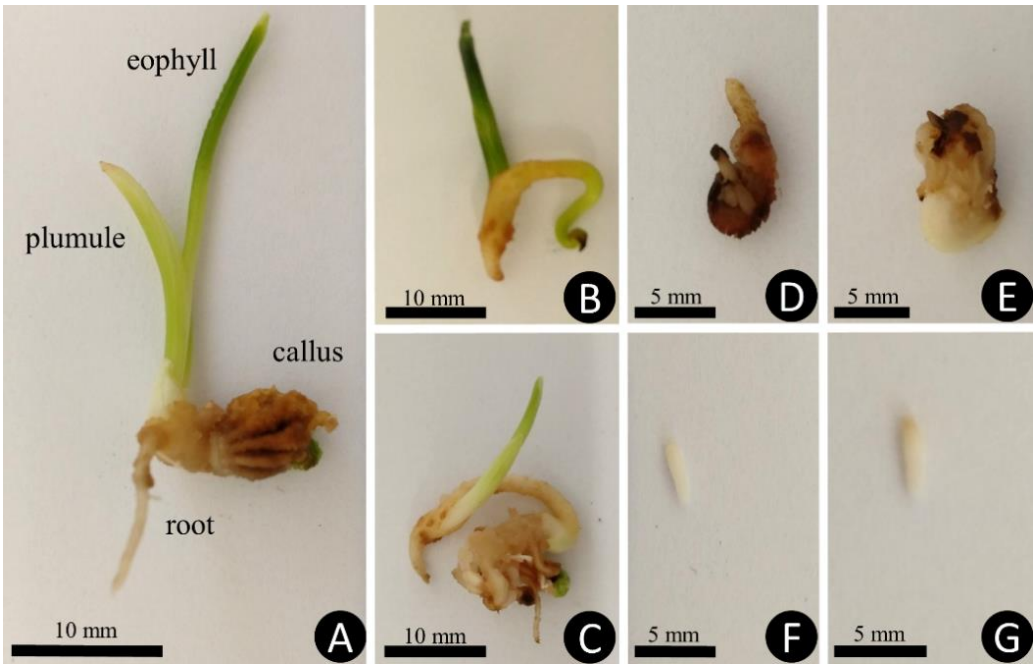

Figure 1. Qualitative classification of the final stage of development of *S. romanzoffiana* embryos: Developed seedlings (A, B and C); dead seedlings (D and E); and non-developed embryos (F and G).

Figura 1. Classificação qualitativa do estado final de desenvolvimento dos embriões de *S. romanzoffiana*: Plântulas desenvolvidas (A, B e C); plântulas mortas (D e E); e embriões não desenvolvidos (F e G).

Results of the dichlorophenoxyacetic acid (2,4-D) experiment

In the experiment with dichlorophenoxyacetic acid (2,4-D), 75% of the explants showed no growth of microorganisms, therefore, they were not contaminated. Based on the masses data of explants classified as 'developed seedlings', analysis of variance (ANOVA) was carried out and the means were compared between treatments. An F_0 of 1.204 and p = 0.350 (F (p<0.05) (3.12) = 3.49) were obtained. The population mean did not demonstrate a significant difference in mass (g) between treatments (Figure 2).

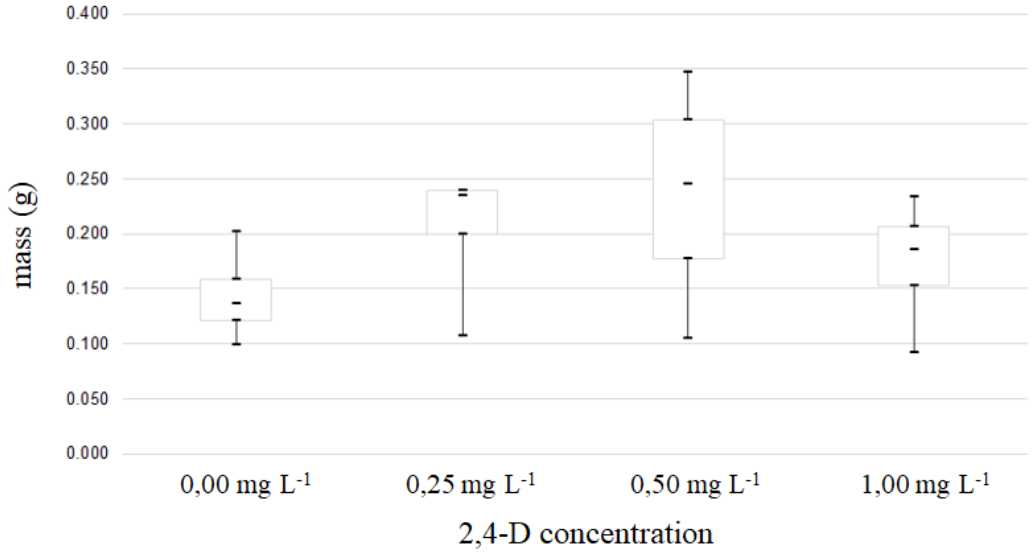

Figure 2. Mass (g) of *S. romanzoffiana* seedlings according to different concentrations of 2,4-D (0.00, 0.25, 0.50, 1.00 mg mL^{-1}).

Figura 2. Massa (g) das plântulas de *S. romanzoffiana* de acordo com diferentes concentrações de 2,4-D (0,00, 0,25, 0,50, 1,00 mg mL^{-1}).

Results of the gibberellic acid (GA_3) experiment

In the experiment with gibberellic acid, 71.2% of the explants showed no growth of microorganisms, a percentage of non-contamination close to the experiment with dichlorophenoxyacetic acid (2,4-D). The mass (g) of explant embryos classified as 'developed seedlings' was weighed for each gibberellin treatment. From analysis of variance (ANOVA) and comparison of seedling mass averages between treatments, an F_0 of 1.687 and p = 0.222 (F (p<0.05) (3.12) = 3.49) were obtained. The population means did not demonstrate a significant difference in mass (g) among treatments (Figure 3).

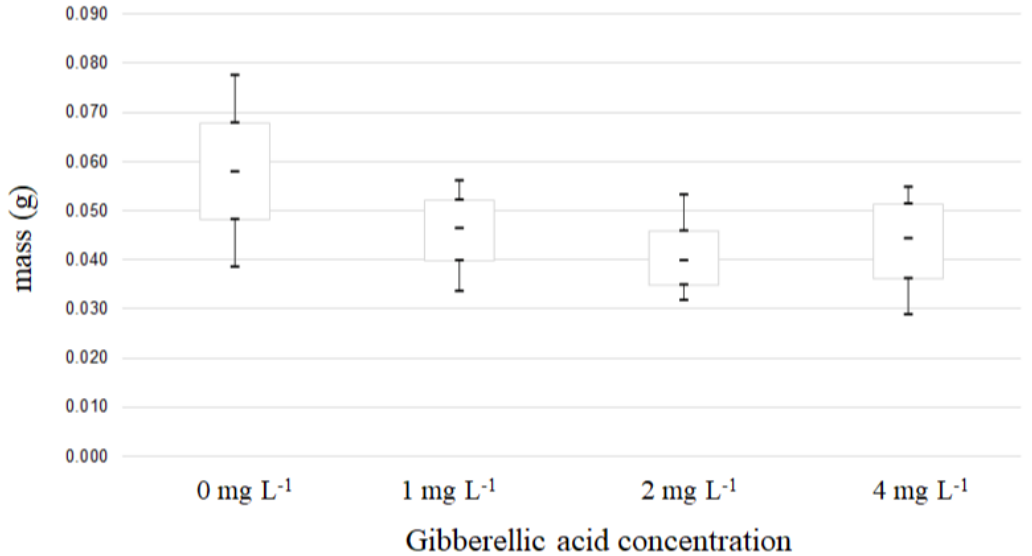

Figure 3. Mass (g) of *S. romanzoffiana* seedlings in four concentrations of gibberellic acid (0, 1, 2 and 4 mg L^{-1}).

Figura 3. Massa (g) das plântulas de *S. romanzoffiana* em quatro concentrações de ácido giberélico (0, 1, 2 e 4 mg L^{-1}).

Analysis of the tetrazolium experiment

The difference between the stained embryos that showed activity in living tissues and the unstained ones was very clear (Figure 4). At 0.25% concentration, 30% of the embryos were non-viable, at 0.50% concentration, 17.5% were non-viable and at the highest concentration, 1.00%, 27.6% of the embryos were non-viable. Thus, on average, 25% of the embryos were non-viable and consequently 75% were viable (Table 1).

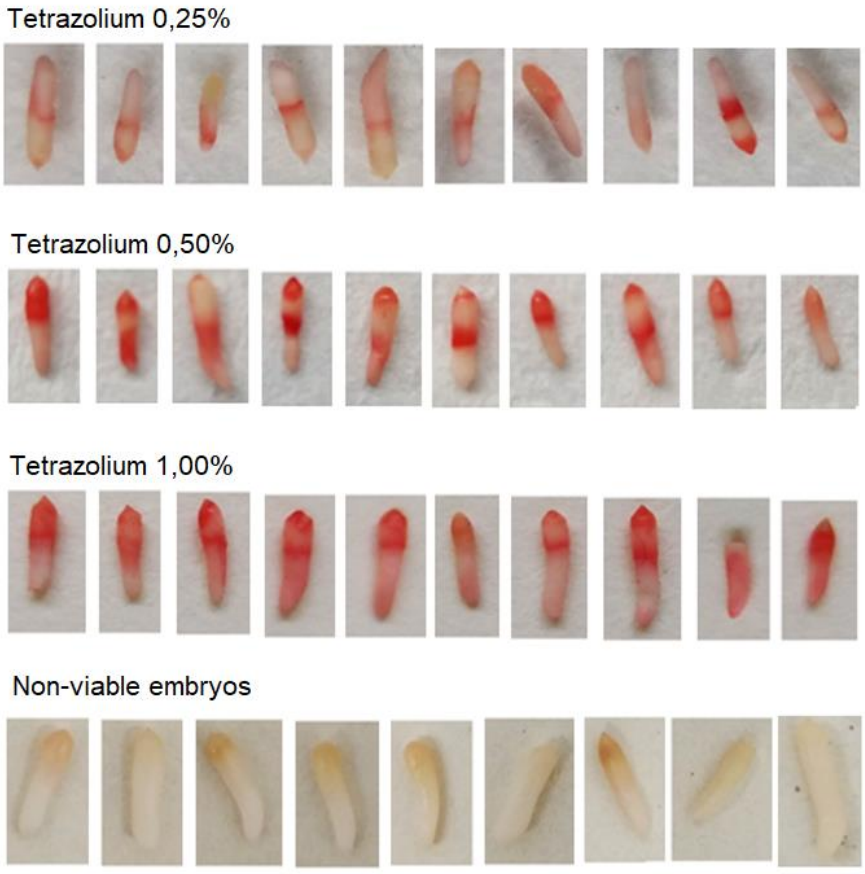

Figure 4. Results of the Tetrazolium test for *S. romanzoffiana* embryos with concentrations of 0.25%, 0.50% and 1.00% after 5 hours. And the final state of non-viable embryos.
Figura 4. Resultados do Teste de Tetrazólio para os embriões de *S. romanzoffiana* com a concentração de 0,25%, 0,50% e 1,00% após 5 horas. E o estado final dos embriões inviáveis.

The tetrazolium test evaluates the potential viability of seeds and embryos. And it is possible to compare the three experiments regarding the percentages of non-viable embryos from the tetrazolium test (25.00%) and the percentages of non-developed embryos from the experiments with 2,4-D (20.63%) and GA_3 (29.38%) (Table 1). The percentages were very close, although, it was not possible to compare the viable embryos from the tetrazolium test with the seedlings developed from the other experiments (2,4-D and GA_3) due to contaminated explants.

Table 1. Percentage comparison of development success and viability of *S. romanzoffiana* embryos.
Tabela 1. Comparação percentual de sucesso do desenvolvimento e viabilidade dos embriões de *S. romanzoffiana*.

Experiment	Developed seedlings	Dead seedlings	Non-developed embryos	Contaminated explants
2,4-D	39,38%	15,00%	20,63%	25,00%
GA_3	25,00%	16,88%	29,38%	28,75%
Tetrazolium	75,00%*		25,00%*	-

*For comparison between the three experiments, it is only possible to compare the "non-viable embryos" in the tetrazolium test with the "non-developed embryos" in the 2,4-D and GA_3 experiments.

DISCUSSION

According to results observed in experiments with dichlorophenoxyacetic acid (2,4-D) and gibberellic acid (GA_3), 32.2% of the inoculated embryos developed and formed seedlings. Ribeiro *et al*. (2011) using in vitro germination of *Butia capitata* (Mart.) Becc. zygotic embryos observed that 26% of the embryos developed into seedlings. In his experiment the use of the embryo disinfection and the time spent without light exposure of 30 days were the main differences from our experiment. The percentage of developed seedlings between the present work and these other two works were very close to each other.

On average, 26.9% of the inoculations resulted in contaminated explants in the experiments with both 2,4-D and GA_3. A low percentage, considering that no embryo disinfection was performed in any of the experiments. Furthermore, 15.9% of the seedlings died during the development without signs of contamination and 25% of the embryos did not even develop, this may have been caused since the fruits were collected under different maturation conditions, which caused the heterogeneity of survival between the embryos.

No significant differences were observed among treatments for each experiment using 2,4-D or GA_3, but a significant difference in mass gain (g) was observed between the use of 2,4-D and GA_3. The average mass for seedlings treated with 2,4-D was 0.189 g, while the average mass for those treated with GA_3 was 0.045 g. That means, the mass gain from seedlings treated with 2, 4-D was at least four times bigger than the mass gain from those treated with GA_3. This difference in mass is caused by the presence of callus in the development of seedlings treated with 2,4-D. We could not provide a suitable explanation for the difference of mass between the two controls (2,4-D at 0 mg/L and GA_3 at 0 mg/L). Furthermore, the absence of a significant difference among the treatments of the two experiments indicates that there is no need to add a germination inducer, resulting in a lower cost of preparing the culture medium. A callus development resulted of disordered cell growth was noticeable for the seedlings treated with 2,4-D, what did not happen in the experiment with GA_3. This is related to the development of calluses in numerous studies using 2,4-D to induce growth of somatic embryos, like in the first work of Halperin and Wetherell (1964) in which a callus was produced from any part of a carrot in order to induce the growth of somatic embryos. Knowledge of callus formation in plants can be used for different studies in microprogramming and also when it is necessary to perform tests on groups of genetically similar plant cells.

In our tetrazolium test conducted at room temperature (25 ± 2°C), we obtained 75% embryo viability among the three concentrations, results are similar to those found in the literature. The work of Iossi *et al*. (2016) evaluated *S. romanzoffiana* embryos with the tetrazolium test, showing 61% of viability among embryos, at a concentration of 0.2% tetrazolium at 40ºC and a maximum period of 6 hours. The work of Oliveira *et al*. (2015) showed 80% initial viability of *S. romanzoffiana* seeds based on the tetrazolium test, at a concentration of 0.5% tetrazolium, at 35ºC, for 4 hours. In addition to each study using a different protocol, such as imbibition time and temperature, to assess the viability of embryos based on the tetrazolium test, there are variables that may justify the differences in results, such as natural differences in the seeds used in each study and the collection and storage conditions.

We noticed that in the tetrazolium test lower concentrations (0,25% and 0,5% tetrazolium), specific regions of the embryo, such as the tips and middle of the embryo, acquired a more intense reddish color (Figure 4). It is possible to distinguish viable (reddish) embryos from non-viable (white) embryos after 3 hours of imbibition and 0.25% tetrazolium, although they do not acquire a reddish color homogeneously. Iossi *et al*. (2016) concluded that a test carried out with 0.2% tetrazolium concentration, at 40ºC for two hours, is adequate to verify the viability of *S. romanzoffiana* embryos. However, in the present work, the test was conducted at room temperature (25º± 2°C) it is possible that a higher temperature could accelerate the imbibition time. Therefore, if carried out at room temperature, we recommended at least 0.5% tetrazolium concentration with five hours imbibition time, for better results.

CONCLUSIONS

- A protocol was established for the *in vitro* germination of *Syagrus romanzoffiana* from the culture of zygotic embryos. The percentage of seedlings formed was 32.2%. And the percentage of contamination was only 26.9%. The seedlings in the experiment with 2,4-D had a much higher mass measurement, caused by the formation of calluses.
- The methodology described using the tetrazolium test to assess embryo viability was proven to be satisfactory. Regarding the imbibition time in tetrazolium, five hours at 0.5% tetrazolium concentration are recommended. With just three hours of imbibition, the embryo coloration is not as intense, which makes the color analysis less clear.

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REFERENCES

BARRUETO CID, L. P.; TEIXEIRA J. B. Explante, meio nutritivo, luz e temperatura. In: BARRUETO CID, L. P. **Cultivo in vitro de plantas**. 4ª ed. Embrapa Recursos Genéticos e Biotecnologia. Brasília - DF. Embrapa, 2015.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Regras para Análise de sementes**. 1 ed. 399 p. Brasília. Mapa/ACS, 2009.

DE SOUZA G. F.; DE ARAÚJO F. F.; FARIAS P. F.; ZANOTTO W. A.; NERI-NUMA A. I.; PASTORE M. G. Brazilian fruits of Arecaceae family: An overview of some representatives with promising food, therapeutic and industrial applications. **Food Research International**, Amsterdam, Netherlands, v. 138, 2020.

DEL POZO, D. G.; MARTÍN-GÓMEZ, J. J.; TOCINO, Á.; CERVANTES, E. Seed geometry in the Arecaceae. **Horticulturae**, Basel, Switzerland, v. 6, n. 4, p. 1–22, 2020.

DIAS, D. S.; LOPES, P. S. N.; RIBEIRO, L. M. Effects of seed structures, sucrose and gibberellic acid on the germination of Butia capitata (Arecaceae). **Seed Science and Technology**, Bassersdorf, Switzerland, v. 41, n. 3, p. 371–382, 2013.

FERREIRA, D. F. SISVAR: A COMPUTER ANALYSIS SYSTEM TO FIXED EFFECTS SPLIT PLOT TYPE DESIGNS. **Brazilian Journal of Biometrics**, Lavras-MG, Brazil, v. 37, n. 4, p. 529–535, 2019.

FRANÇA-NETO, J. DE B.; KRZYZANOWSKI, F. C. Metodologia do teste de tetrazólio em sementes de soja Metodologia do teste de tetrazólio em sementes de soja. **Embrapa Soja (Documento 406)**, Londrina-PR, Brazil, p. 109, 2018.

FRÖHLICH R.; GUIMARÃES V. F.; JUNIOR R. C.; BRITO T. S.; SILVA A. S. L.; VITTO D. C.; ANKLAN M. A. Desenvolvimento e produção de plantas de soja tratadas com ácido 2,4 diclorofenoxiacético em subdosagem. **Cultivando o Saber**. Cascavel-PR, Brazil, p. 138, v. 14, 2021.

HALPERIN W.; WETHERELL D. F. Adventive embryony in tissue cultures of the wild carrot, Daucus carota. **American Journal of Botany**, St. Louis, USA, v. 51: 274-283, 1964.

IOSSI, E.; MORO V. F.; VIEIRA LICURSI T. G. B.; BARBOSA M. R.; VIEIRA D. R. Chemical Composition and Tetrazolium Test of Syagrus romanzoffiana (Cham.) Glassman Seeds. **Revista Brasileira de Fruticultura [online]**. Jaboticabal-SP, Brazil, v. 38, n. 4, 2016.

JORGE, N.; DA SILVA, A. C.; VERONEZI, C. M. Syagrus romanzoffiana. In: FREITAS DE LIMA, F., LESCANO, C.H., PIRES DE OLIVEIRA, I. (eds) **Fruits of the Brazilian Cerrado**. Springer, Cham, Switzerland. p. 141-159, 2021.

MINARDI, B. D.; VOYTENA, A. P. L.; RANDI, A. M.; ZAFFARI, G. R. Cultivo in vitro de embriões zigóticos de Butia eriospatha (Mart. Ex Drude) Becc. **Insula**, Florianópolis-SC, Brazi, ln. 40, p. 70-81, 2011.

MURASHIGE, T.; SKOOG, F. A. A revised medium for rapid growth and bioassays with tabaco tissue cultures. **Physiologia Plantarum**, Copenhagen, Denmark, v. 15, p. 473-497, 1962.

NETO, A. R.; MACHADO, C. A.; CHAGAS, E. A.; COSTA S. N. B.; CHAGAS C. P.; VENDRAME A. W. In vitro germination of Syagrus romanzoffiana (Cham.) Glassman. **Plant Cell Culture & Micropropagation**, Lavras-MG, Brazil, v. 16, p. 1–6, 2020.

NOBLICK L. R. A revision of the genus Syagrus (Arecaceae). **Phytotaxa**, Auckland, New Zealand, v. 294, n. 1, 2017.

NOBLICK L. R. Syagrus guaratingensis: a new species from Bahia, Brazil. **Palms**. Lake Worth, USA, 62(2): 77–86, 2018.

OLIVEIRA, T. G. S.; JOSÉ, A. C.; RIBEIRO, L. M.; ROCHA FARIA, J. M. Longevity and germination of Syagrus romanzoffiana (Arecaceae) seeds and its ecological implications. **Revista de Biología Tropical**, San José, Costa Rica, v. 63, n. 2, p.333-340, 2015.

PEDÓ T.; MARTINAZZO G. E.; BACARIN A. M.; ANTUNES F. I.; KOCH F.; MONTEIRO A. M.; PIMENTEL R. J.; TROYJACK C.; FVILLELA A. F.; AUMONDE Z. T. Crescimento de plantas e vigor de sementes de feijão em resposta à aplicação exógena de ácido giberélico. **Revista de Ciências Agrárias**, Belém-PA, Brazil, v. 41, n. 3, p. 757–770, 2018.

REGO, C. H. Q.; CARDOSO, F. B.; COTRIM, M. F.; CÂNDIDO, A. C. DA S.; ALVES, C. Z. Ácido Giberélico Auxilia Na Superação Da Dormência Fisiológica E Expressão De Vigor Das Sementes De Graviola. **Revista De Agricultura Neotropical**, Cassilândia-MS, Brazil, v. 5, n. 3, p. 83–86, 2018.

RIBEIRO, L. M.; NEVES, S. C.; SILVA, P. O.; ANDRADE, I. G. Germinação de embriões zigóticos e desenvolvimento in vitro de coquinho-azedo. **Revista Ceres**, Viçosa-MG, Brazil, v. 58, n. 2, p. 133-139, 2011.

SANT'ANNA-SANTOS B. F.; AZEVEDO I. F. P.; MICHELI R.; SOFFIATTI P. Morpho-anatomical novelties of a dwarf Syagrus (Arecaceae) of canga: implications for ecology, conservation, and taxonomy. **Plant Systematics and Evolution**, Vienna, Austria, 309, 8, 2023.

SOARES K. P.; GUIMARÃES C. Syagrus amicorum, a new Arecaceae from Bahia, Brazil. **Phytotaxa**. Auckland, New Zealand, 387(2): 158–164, 2019.

SOARES, K. P. Syagrus in Flora e Funga do Brasil. **Jardim Botânico do Rio de Janeiro**, Rio de Janeiro-RJ, Brazil 2023. Disponível em: <https://floradobrasil.jbrj.gov.br/FB15745>. Acesso em: 27 fev. 2023.

SOUSA, D. M. M.; BRUNO, R. DE L. A.; SILVA, K. DA R. G.; TORRES, S. B.; ANDRADE, A. P. Viabilidade e vigor de sementes de Poincianella pyramidalis (Tul.) L. P. Queiroz pelo teste de tetrazólio. **Revista Ciencia Agronomica**, Fortaleza, CE, Brazil, v. 48, n. 2, p. 381–388, 2017.

TOFANELLI, M. B. D.; FREITAS, P. L.; PEREIRA, G. E. 2,4-Dichlorophenoxyacetic Acid as an Alternative Auxin for Rooting of Vine Rootstock Cuttings. **Revista Brasileira de Fruticultura**, Jaboticabal, SP, Brazil, v. 36, n. 3, p. 664–672, 2014.