BIOSCIENCE JOURNAL

IN VITRO ESTABLISHMENT AND MULTIPLICATION OF *Echynochloa polystachya*

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How to cite: SILVEIRA, E.O., et al. *In vitro* establishment and multiplication of *Echynochloa polystachya*. *Bioscience Journal*. 2022, **38**, e38075. https://doi.org/10.14393/BJ-v38n0a2022-60508

Abstract

The objective of this work was to carry out the *in vitro* establishment of *Echynochloa polystachya* aiming at obtaining a micropropagation protocol for works involving the selection of superior genotypes and the cultivation of the species. *E. polystachya* stems were collected in the municipality of Manaus-AM. Explants were inoculated in test tubes containing Murashige and Skoog (MS) medium. Thirty days after *in vitro* establishment, the rate of sprouting and contamination were evaluated. Experiments were also carried out to assess the effects of sucrose and 6-benzylaminopurine (BAP) concentrations on the tillering rate of explants. It was found that during the successive subcultures there was a decrease in internodes and the consequent loss of vigor. There were responses in the multiplication rate at concentrations starting from 45 g L^{-1} sucrose. In addition, BAP and sucrose interfered the development and *in vitro* multiplication. Sucrose in conjunction with BAP was harmful and shortened internodes. The physiological state of the explants for the species under study was intrinsically linked to the concentrations of sucrose used for the culture medium and the concentrations of BAP. However, the sucrose and BAP concentrations suggested for *in vitro* cultivation of *E. Polystachya* must be adjusted during successive subcultures. Absence of contamination in the *in vitro* establishment occurred at concentrations 15, 30 and 60 g L^{-1} sucrose. The combination of 1.5 mg L^{-1} BAP and 30 g L^{-1} sucrose promoted greater induction of sprouts. In addition, the *in vitro* rooting of *E. polystachya* was 45%.

Keywords: Creeping River Grass. Micropropagation. Plant Growth Regulators. Tissue Culture.

1. Introduction

In vitro propagation or micropropagation can be defined as a process by which plant fragments (explants) are grown in aseptic conditions in a culture medium under appropriate conditions that enable the multiplication and regeneration of plants complete and genetically identical to the original organism (Amaral and Silva 2003). A plant grown *in vitro* has its heterotrophic metabolism modified and dependent on water, macro and micronutrients and carbohydrates from the culture medium for carbon synthesis (Pierik 1988).

However, the conditions suitable for micropropagation vary between species, genotypes of the same species and also according to the explant used.

Given the possibility of obtaining complete plants from the culture of tissues or plant organs, micropropagation presents itself as an important tool in plant breeding, since in addition to mass multiplication, saving time and space, it is possible to obtain individuals with genetic characteristics identical to the original matrix, in addition to the fact that it is an interesting and efficient strategy for plant conservation (Pence 2011).

Creeping river grass (*Echinochloa polystachya*) is an excellent alternative as food in livestock production in the Amazon region, given its intense growth and good acceptance, especially by buffaloes and cattle. Despite its great importance for the Amazon region (Barbosa et al. 2008), there is still little information in the literature about the species, such as conditions for its establishment on dry land, propagation, planting management, genetic variability, agronomic performance and grazing production.

Thus, the establishment of a protocol for the micropropagation of *E. polystachya* can contribute to rapid multiplication of genotypes of interest, for example, genotypes selected for high production and palatability, regeneration and multiplication of genotypes subjected to mutagenic treatments, experiments with transformation of plants, among other studies that require plants obtained in aseptic conditions. However, to date, there are no records of studies for the development of micropropagation protocols for creeping river grass.

Considering the potential of micropropagation application for the culture of creeping river grass, the objective of this work was to develop an efficient protocol for *in vitro* cultivation of the species.

2. Material and Methods

Obtaining explants and *in vitro* establishment

For the installation of the experiments, a whole stem of creeping river grass was collected in the municipality of Manaus-AM (3°06'56.3"S 60°02'01.9"W). The collected stem was planted in pots and kept in a nursery at Embrapa Amazônia Ocidental to maintain the genotype to be used in the experiments.The explants were removed for *in vitro* inoculation after the emission of tillers, using tillers containing 3 to 4 knots each. The tillers were sectioned and taken to the Plant Tissue Culture Laboratory of Embrapa Amazônia Ocidental, where they passed through the initial pre-cleaning and disinfestation phase, removed the leaf sheath and senescent material and obtained the nodal segments that were used as explants for the *in vitro* establishment of the genotype.

After the initial phase, the nodal segments were transferred to the laminar flow chamber, previously sterilized (UV light), where they were subjected to the disinfestation process as proposed by Passos and Köpp (2010) (adapted), consisting of immersion in alcohol 70 % for 2 min, followed by 50% calcium hypochlorite solution ($CaCl_2$ O_2) for 15 min.

After disinfestation, the explants were washed three times in autoclaved distilled water. Then, with the help of scalpels and tweezers, the nodal segments were reduced to 1.0 cm in size and individually inoculated in test tubes measuring 150 mm x 25 mm containing 15 mL of the Murashige and Skoog (MS) culture medium (Murashige and Skoog 1962).

In establishing the explants *in vitro*, the MS culture medium was used with 50% of the concentration of mineral salts of the original composition (MS-50), plus 4.5 mg L^{-1} 6-benzylaminopurine (BAP), 30 g L^{-1} sucrose, 2 mL L^{-1} PPM biocide and 2 g L^{-1} phytagel solidifying agent. The pH was adjusted to 5.8 before autoclaving.

After inoculation, the material was kept in the dark covered with black plastic to avoid incidence of light and temperature of 25 ± 2 °C for eight days. Then, the tubes were maintained in a growth room with a photoperiod of 16 h and irradiance of 90 to 110 µmol m^{-2} s^{-1}, conditions in which they remained until completing 30 days.

In vitro multiplication

After the *in vitro* establishment phase, the explants moved to the multiplication phase in order to induce sprouts in the explants.

In the multiplication phase, the MS medium with integral concentration of mineral salts (MS-100) was used, plus 4.5 mg L^{-1} BAP, 30 g L^{-1} sucrose, 2 mL L^{-1} PPM and variations in sucrose, BAP and phytagel concentrations, four experiments were carried out separately. The pH was adjusted to 5.8 before autoclaving.

During the multiplication phase, the material was subculture every 20 days, when the sprouts were separated and inoculated in fresh culture medium with the same composition and the number of sprouts produced per explant and the percentage of contamination were counted.

Effect of sucrose concentration on *in vitro* multiplication

The explants were subjected to six concentrations of sucrose (0, 15, 30, 45, 60 and 75 g L^{-1}) in MS medium (MS-100), plus 4.5 mg L^{-1} BAP, 2 mL L^{-1} PPM and 2 g L^{-1} phytagel. The pH of the medium was adjusted to 5.8. The explants were cultured individually in test tubes with dimensions of 150 mm x 25 mm containing 15 mL of medium. The tubes were kept in a growth room with a temperature of 25 ± 2 °C, a photoperiod of 16 h and irradiance of 90 to 110 µmol m^{-2} s^{-1}.

The experimental design was completely randomized with six treatments and five replications. Each repetition was represented by five explants, for a total of 25 explants per treatment. After 30 days of *in vitro* cultivation, number of sprouts produced per explant and percentage of contamination were evaluated.

The data were subjected to analysis of variance and the means compared by the Tukey´s test at 5% probability. Statistical analyzes were performed using RBIO, a program integrated with the R environment (Bhering 2017). The tests for data normality analysis were verified with the Shapiro-Wilk and Lilliofers tests using the GENES version 6.1 program (Cruz 2013).

Effect of BAP on the multiplication of explants

The explants were cultured in MS-100 culture medium, plus 30 g L^{-1} sucrose, 2 mL L^{-1} PPM and 2 g L^{-1} phytagel, with five concentrations of BAP (0.0; 1, 5, 3.0, 4.5 and 6.0 mg L^{-1}). The pH of the medium was adjusted to 5.8. The explants were cultured individually in test tubes with dimensions of 150 mm x 25 mm containing 15 mL of medium. The tubes were kept in a growth room at 25 ± 2 °C under a photoperiod of 16 h and irradiance of 90 to 110 µmol m^{-2} s^{-1}.

The experimental design was completely randomized with five treatments and five replications. Each repetition represented by five explants, for a total of 25 explants per treatment. After 30 days of *in vitro* cultivation, the number of tillers was evaluated.

The data were subjected to analysis of variance and the means compared by Tukey's test at 5% probability and regression analysis. Statistical analyzes were performed using RBIO, a program integrated with the R environment (Bhering 2017). The tests for data normality analysis were verified with the Shapiro-Wilk and Lilliofers tests using the GENES version 6.1 program (Cruz 2013).

Effect of BAP and sucrose on the sprouting of explants

The explants were subjected to five concentrations of BAP (0.0; 1.5; 3.0; 4.5 and 6.5 mg L^{-1}) and two concentrations of sucrose (30 and 60 g L^{-1}) in MS culture medium (MS-100), plus 1 mL L^{-1} PPM and 2 g L^{-1} phytagel and without plant growth regulators (PGR). The pH of the medium was adjusted to 5.8. The explants were cultured individually in test tubes with dimensions of 150 mm x 25 mm containing 15 mL of medium. The tubes were kept in a growth room at 25 ± 2 °C under a photoperiod of 16 h and irradiance of 90 to 110 µmol m^{-2} s^{-1}.

The experimental design was completely randomized in factorial schema 5 (BAP concentrations) x 2 (sucrose concentrations) with two replicates. Each repetition represented by five explants, for a total of 10

explants per treatment. After 30 days of *in vitro* cultivation, the number of sprouts produced per explant was evaluated.

The data were submitted to analysis of variance and the means compared by Tukey's test at 5% probability and regression analysis. Statistical analyzes were performed using RBIO, a program integrated with the R environment (Bhering 2017). The tests for data normality analysis were verified with the Shapiro-Wilk and Lilliofers tests using the GENES version 6.1 program (Cruz 2013).

Effect of phytagel concentration on the sprouting rate of explants

The explants were grown in MS culture medium plus 4.5 mg L^{-1} BAP, 30 g L^{-1} sucrose and 2 mL L^{-1} PPM, combined with two concentrations of phytagel (1.4 and 2.0 g L^{-1}). The pH of the medium was adjusted to 5.8. The explants were cultured individually in test tubes with dimensions of 150 mm x 25 mm containing 15 mL of medium. The tubes were kept in a growth room at 25 ± 2 °C under a photoperiod of 16 h and irradiance of 90 to 110 µmol m^{-2} s^{-1}.

The experimental design was completely randomized with two treatments and five replications. Each repetition represented by five explants, for a total of 25 explants per treatment. After 30 days, the sprouting rate was evaluated.

The data were subjected to analysis of variance and the means compared by the Tukey´s test at 5% probability and regression analysis. Statistical analyzes were performed using RBIO, a program integrated with the R environment (Bhering 2017). The normality analysis tests of the data were verified with the Shapiro-Wilk and Lilliofers tests using the GENES version 6.1 program (Cruz 2013).

Rooting of explants multiplied *in vitro*

In the rooting phase, 144 explants originating from the sprouts of the experiments with variation in the concentrations of BAP and sucrose were used. In this phase, we used the medium MS-50, plus 30 g L^{-1} sucrose, 2 mL L^{-1} PPM, 2 g L^{-1} phytagel and 2.25 g L^{-1} activated charcoal and without PGR. The pH was adjusted to 5.8. The explants were cultured individually in test tubes with dimensions of 150 mm x 25 mm containing 15 mL of medium.

After inoculation of the material in the rooting medium, the tubes were kept in a growth room at 25 ± 2 °C under a photoperiod of 16 h and irradiance of 90 to 110 µmol m^{-2} s^{-1}. At the end of this phase, the rooting rate was evaluated.

3. Results

The plant material was grown *in vitro* for 14 months (establishment and multiplication phase), with subcultures approximately every 20 days. The highest occurrence of contamination (25.74%) during the period occurred in the third subculture (Figure 1).

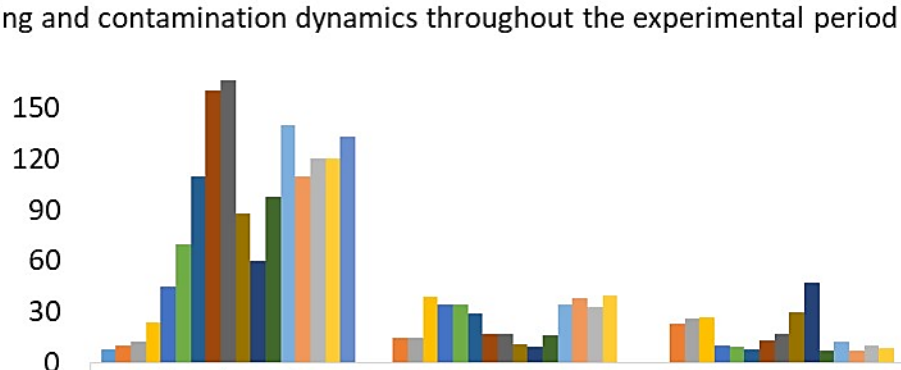
The use of 60 g L^{-1} sucrose exceeded all other concentrations, with an average production of 14 sprouts per explant (Figure 2A). On the other hand, no sprouts were observed in the absence of sucrose. Thus, it can be inferred that concentrations above 60 g L^{-1} are unfavorable to the sprouting rate, since with 75 g L^{-1} the number of sprouts was lower than other concentrations, except in the absence of sucrose.

However, 100% of the explants contaminated in the absence of sucrose in the culture medium. At concentrations 15, 30 and 60 g L^{-1} sucrose, contamination was not observed, and in 45 and 75 g L^{-1} sucrose the contamination rate was 10%. The results with the different sucrose concentrations used demonstrate the importance of the external carbon source for increasing the number of sprouts, which is desired when propagating *in vitro*. In the absence of sucrose in the medium, there was no sprouting.

Thus, the increase in sucrose content was essential for the maintenance and formation of new sprouts, which attest to the need for carbohydrate input in seedlings grown *in vitro* considering the heterotrophic characteristic of the system. However, in the absence of sucrose, the contamination rate was 100% (Figure 2B), which implies the fact that explants die due to the lack of sucrose and subsequently contamination, when compared to the significant decrease in contamination in treatments with the

presence of sucrose. The results obtained in the present study demonstrate that BAP is an important sprout inducer when associated with concentrations of other biochemically active products for *E. polystachya*.

In the combination of five different concentrations of BAP and sucrose (30 and 60 g L^{-1}), only BAP had a significant effect on the rate of explant multiplication. The concentration 1.5 mg L^{-1} BAP surpassed all others, with a sprouting rate of 100%, while in the absence of BAP, no explants showed budding, which was inferior to the other treatments (Figure 3A). The sprouting rate when 60 g L^{-1} sucrose was used was higher in treatments with concentrations of 3.0 to 6.0 mg L^{-1} BAP.



	Number of explants	Sprouts (%)	Contamination (%)
05/07/2018	8	0.00	0.00
24/07/2018	10	14.67	22.92
30/08/2018	12	14.90	25.74
06/11/2018	24	38.60	26.95
19/11/2018	45	34.37	10.40
12/12/2018	70	34.00	9.40
28/12/2018	110	29.00	8.20
22/01/2019	160	17.00	13.00
14/02/2019	166	16.67	17.00
13/03/2019	88	11.00	29.50
05/04/2019	60	9.00	47.30
22/04/2019	98	16.00	7.00
20/05/2019	140	34.00	12.50
28/06/2019	110	37.80	7.27
25/07/2019	120	32.50	10.00
14/08/2019	120	39.70	8.50
14/09/2019	133	0.00	0.00

Figure 1. Number of explants, percentage of sprouting and contamination in *in vitro* cultivation of *E. polystachya*.

However, the contamination rates were higher in the culture medium with 30 g L^{-1} of sucrose, deducing the importance of sucrose in the culture medium when the plant is stimulated to grow in contrast to the culture medium with 60 g L^{-1} sucrose, where contamination was greatest. It is interesting to note that the seedlings remained stagnant, without growth and over time, they were senescent in medium without BAP and with the presence of 60 g L^{-1} sucrose.

Contamination rates were higher at reduced concentrations of BAP when the medium had 30 g L^{-1} sucrose and without BAP (Figure 3B). In the medium with 60 g L^{-1} sucrose, different from that observed with 30 g L^{-1}, the lowest contamination rate (0%) was observed in the treatment without BAP, but presenting

higher rates from 3.0 mg L^{-1} BAP in the culture medium (Figure 3B). Thus, it is possible to observe the importance of sucrose in the culture medium when the plant is stimulated to grow in contrast to the culture medium that contained 60 g L^{-1} sucrose, where the contamination was greatest.

For number of tillers, in the regression analysis it was found that the quadratic model (R^2=1) was adequate to represent the response of the variable as a function of the variation in BAP concentration (Figure 4). According to the equation, the maximum number of tillers was estimated with 4.5 mg L^{-1} BAP.

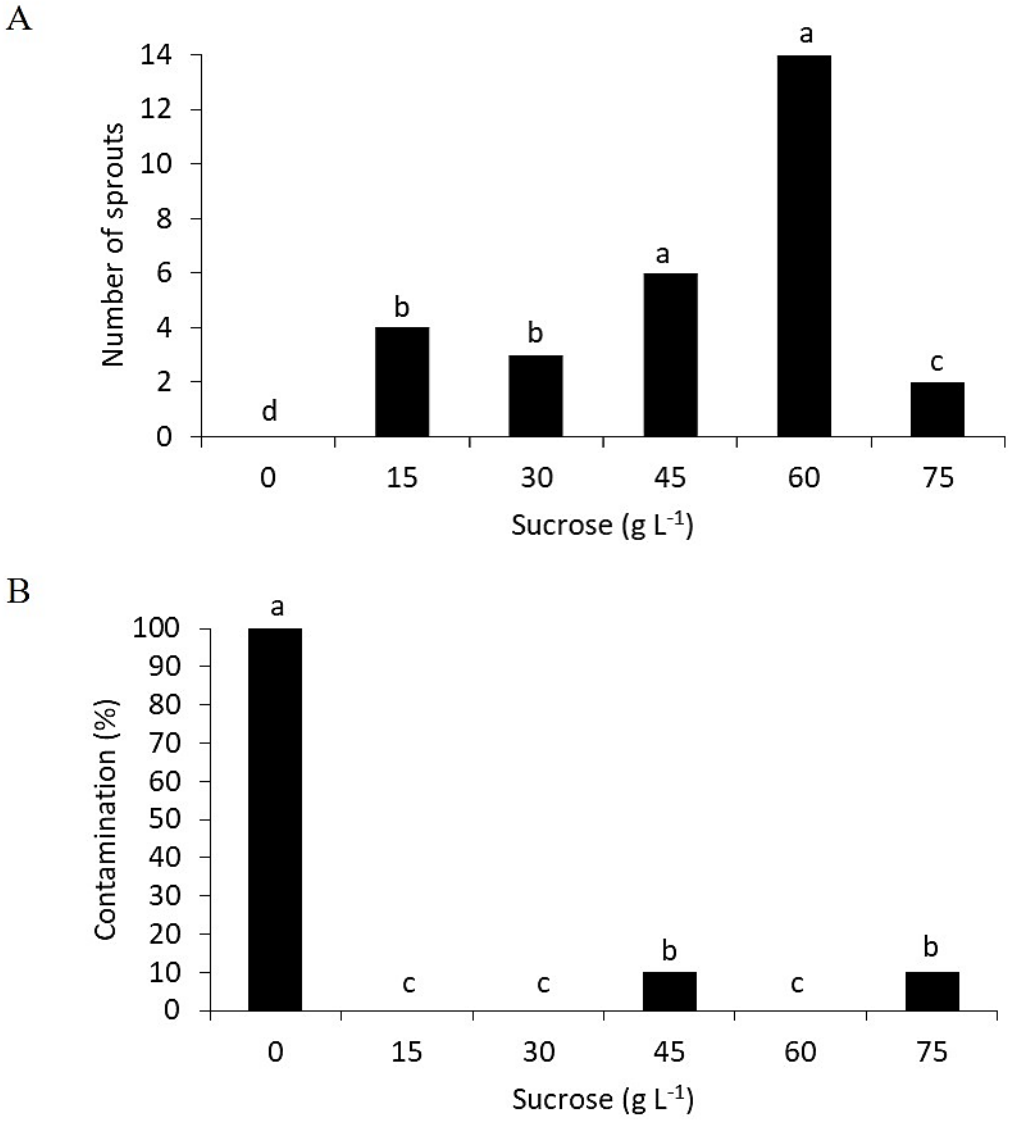


Figure 2. A - Sprouting rates and B - contamination of *Echynochloa polystachya* explants submitted to different sucrose doses in MS culture medium. Averages followed by the same letter do not differ by Tukey's test at 5% probability.

The results obtained in the present study demonstrate that BAP is an important sprout inducer when associated with concentrations of other biochemically active products for *E. polystachya*.

The sprouting rate of the explants was not affected by the variation in phytagel concentration. However, the rate obtained in the MS-100 + 2.0 g L^{-1} (35.65%) did not differ significantly from the MS-100 + 1.4 g L^{-1} (38.80%) (Figure 5).

The concentrations of BAP and sucrose interfered in the development and *in vitro* multiplication of creeping river grass. However, other tests using other PGR, such as naphthalene acetic acid (NAA), may complement information for the *in vitro* multiplication of the species.

In the rooting phase, 144 explants originated from the sprouts obtained in the BAP and sucrose experiments were used. This enabled the rooting rate to be 45%.

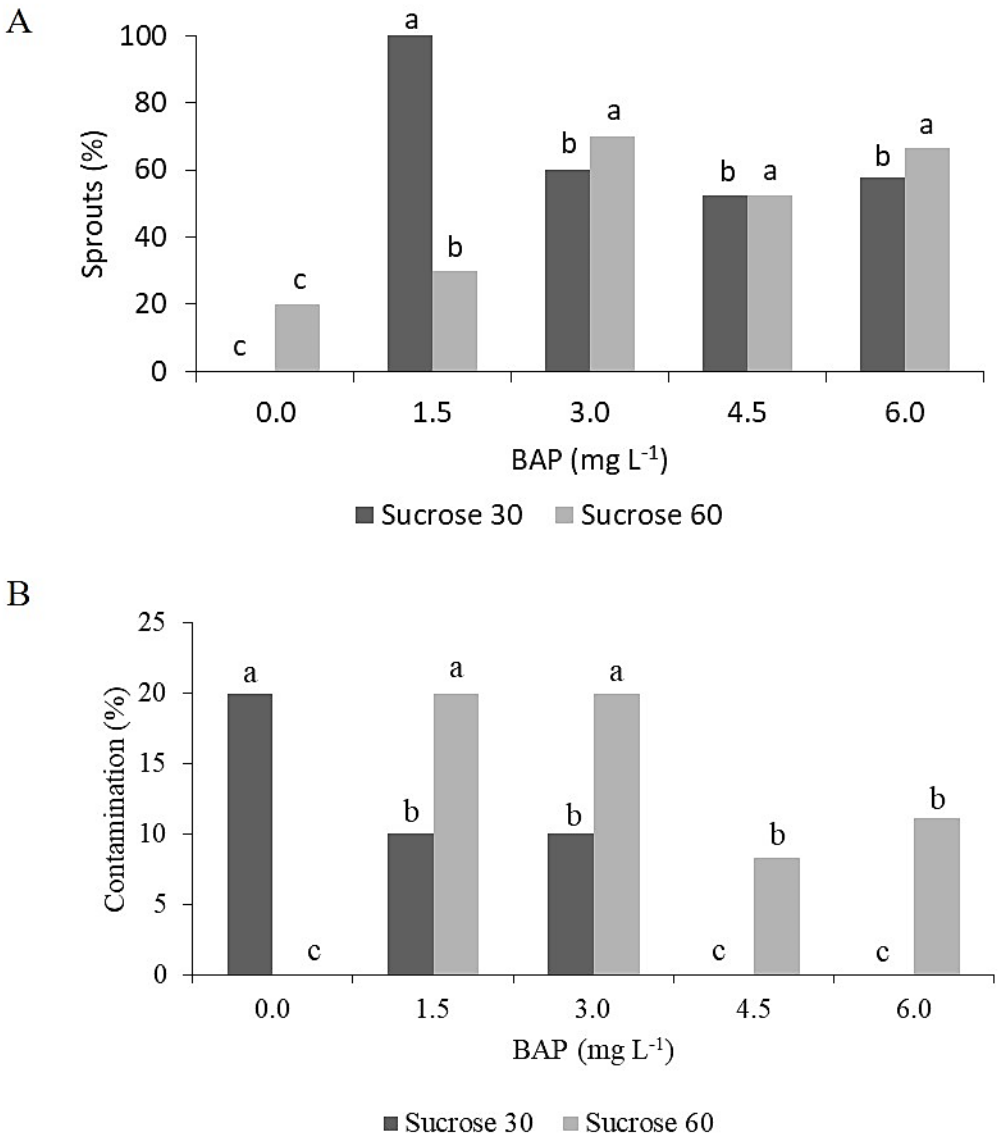


Figure 3. A - Sprouts and B - contamination of *E. polystachya* explants submitted to different concentrations of BAP in MS culture medium with 30 and 60 g L^{-1} sucrose. Means followed by the same letter do not differ by Tukey's test at 5% probability.

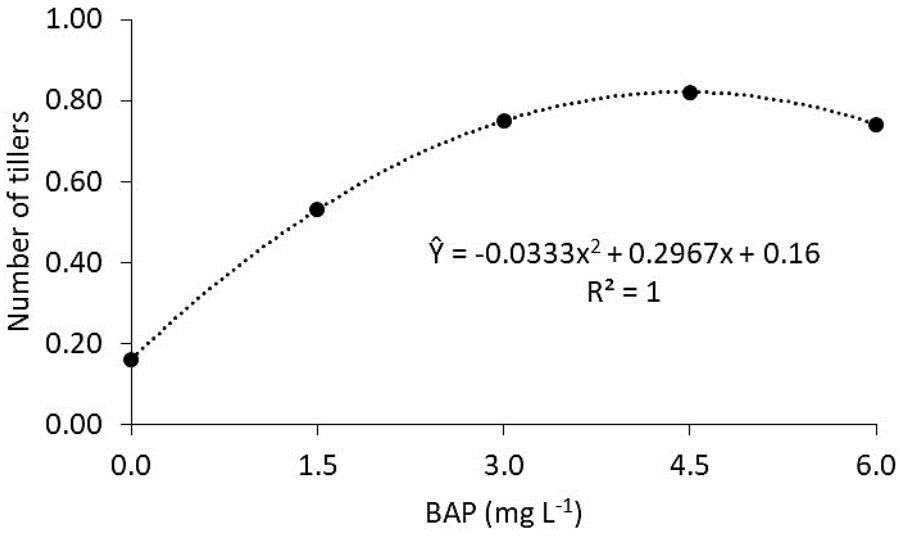


Figure 4. Number of *E. polystachya* tillers as a function of different concentrations of BAP.

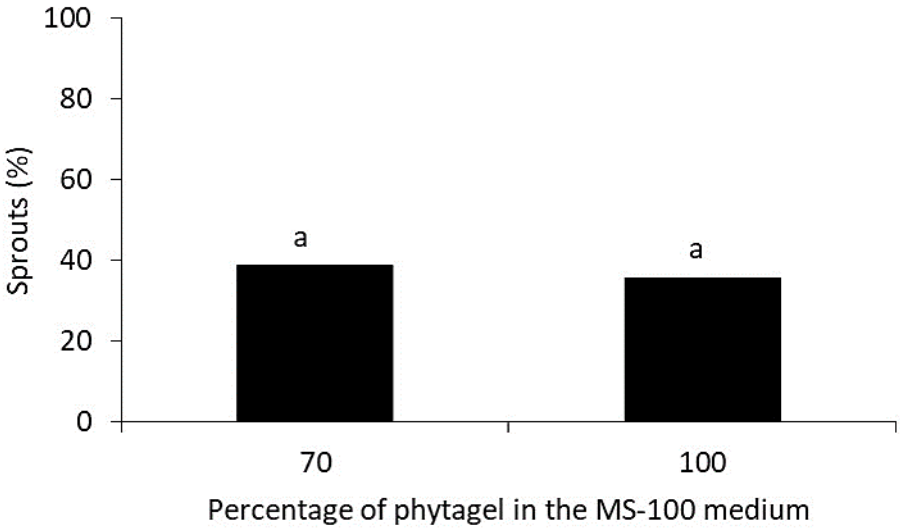


Figure 5. Sprouts of *E. polystachya* at different concentrations of the MS culture medium and the reduction of phytagel concentration by 30%. Means followed by the same letter do not differ by Tukey's test at 5% probability.

The results obtained allowed to establish an initial protocol for the *in vitro* propagation of *E. polystachya*, however, further studies with other variations in the composition of the culture medium must be carried out in order to obtain a high efficiency protocol. In Figure 6, it is possible to observe the *in vitro* cultivation stages of *E. polystachya*.

4. Discussion

The contamination that occurred in the first subculture (July 24, 2019) can be caused by faults in the asepsis of the material and also due to manipulation (Figure 1). From the elimination of the contaminated material in the subsequent subcultures, it is expected that the contamination originates only from handling problems. Thus, contamination was greater in the first subcultures (*in vitro* establishment).

During the period of *in vitro* establishment, sprouts also appeared, comparing the effect of the concentration of mineral salts proposed by Murashige and Skoog (1962). In cultivation with full composition of the MS-100 medium, the sprouting rate was 35.65%, while with MS-50 it was 14.78%.

Over successive subcultures, it was observed that the explants began to lose their vigor considerably. The relationship between loss of vigor and morphological behavior *in vitro* is complex and is influenced by several physiological and environmental factors that can affect the supply of nutrients and growth regulators and biosynthesis. The morphogenic responses of grasses to *in vitro* cultivation are the most varied. This fact is reported by Vidigal et al. (1998) that to verify the increase in vigor in the *in vitro* conservation of elephant grass introduced axillary meristems repeatedly added or not to the medium with 0.125 µM of NAA (naphthalene acetic acid that can provide plant regeneration through meristems. Vidigal et al. (1998) evaluated 51 cultivars, but only one (common Mercker cultivar) did not show a satisfactory response, so the addition of NAA can increase and maintain the vigor of explants during successive subcultures.

For the *in vitro* cultivation of *Desmodium incanum* Maldaner et al. (2014) concluded that the different concentrations of sucrose (0; 15; 30 and 45 g L^{-1}) did not influence the height of the seedlings or the number of roots. However, the concentration of 15 g L^{-1} sucrose favored the emission of sprouts, number of leaves and seedling biomass production, verifying that this sucrose concentration is suitable for *in vitro* cultivation of the species.

The effect of high concentrations of sucrose in the culture medium can also be harmful to seedlings, decreasing the emission rates of new sprouts and affecting the absorption of nutrients due to increased oxidation of the culture medium (Thorpe et al. 2008). Lemos et al. (2002) used apical buds of fourth-cropped sugarcane sprouts and observed the effect of temperature, carbon sources (sucrose, mannitol and sorbitol), osmotic regulators and abscisic acid in the *in vitro* conservation of sugarcane germplasm sugar. In addition,

the lower concentrations of sucrose (10 and 20 g L^{-1}) in the culture medium limited the growth of explants, but maintained the best viability and the lowest percentages of oxidation of the media, providing greater longevity for sugarcane explants. However, the 40 g L^{-1} sucrose concentration showed a high percentage of oxidation of the culture medium and the maintenance of explants viability was significantly reduced.

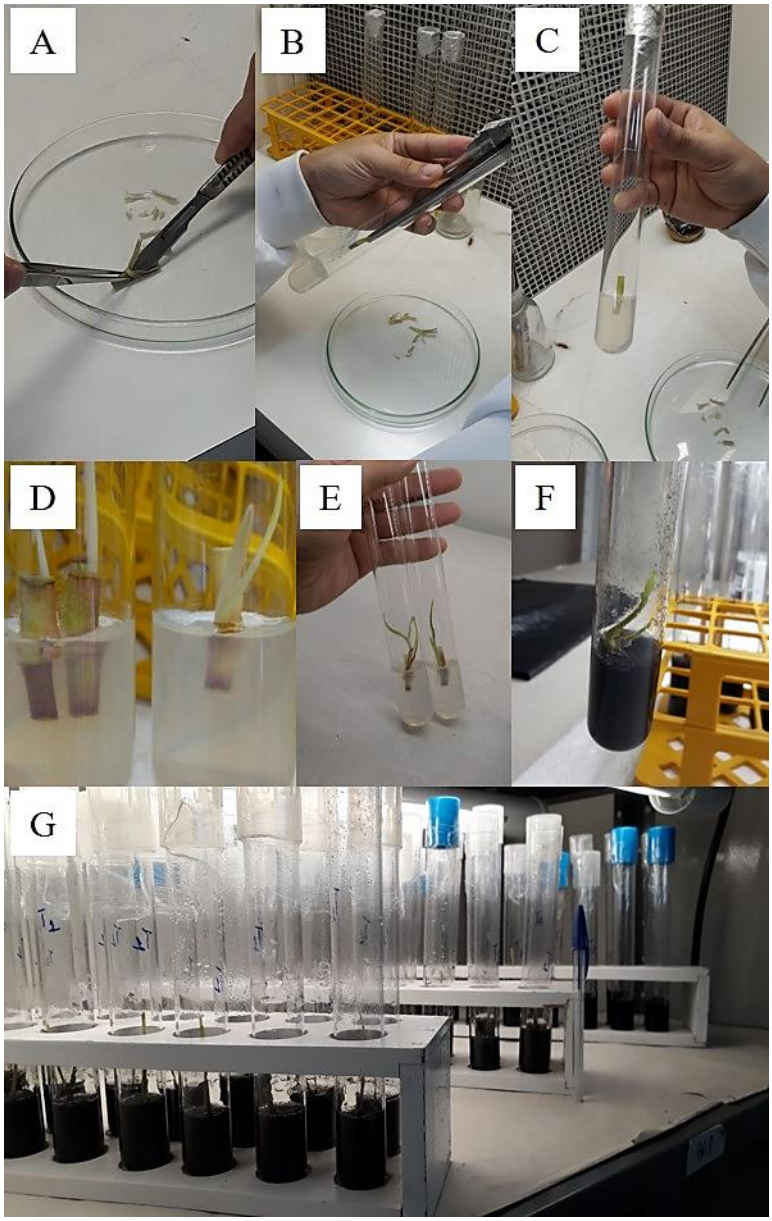


Figure 6. *In vitro* cultivation stages of *E. polystachya*. A to C - Explant preparation and *in vitro* inoculation; D and E - *In vitro* multiplication stage; F and G - *In vitro* rooting stage.

Lemos et al. (2002) also observed better results in treatments in which there was a combination of lower temperatures, suggesting that the joint treatment of high temperatures and sucrose concentration is detrimental to the survival of explants because high sucrose concentrations are absorbed more quickly, increasing the leaf mass and consequent senescence of explants. In addition, high concentrations of sucrose promote oxidation of the culture medium and deterioration of the cultures due to the expressive osmotic potential of the medium (Fossard et al. 1978; Lemos et al. 2002).

Lemes et al. (2016) studied the initial growth of orchid (*Miltonia flavescens*) cultivated in four culture media with different sucrose concentrations (25, 30, 35, 40 and 45 g L^{-1}) and observed that the fresh mass content and the emission of roots explants were influenced by sucrose concentrations when compared to cultivation time. In addition, they observed that concentrations between 20 to 25 g L^{-1} favored the largest amount of fresh mass of the aerial part. In the present study for *E. polystachya* it was found that concentrations higher than these in cultivation medium (for orchids) can interfere in the reduction of the absorption of salts and in the consequent growth of the plant.

Frota et al. (2004) used benzylaminopurine (BAP) and indolacetic acid (IAA) in the culture medium for the propagation of forage palm and found greater shoot induction with 2.00 mg L^{-1} BAP + 0.25 mg L^{-1} IAA, inducing from 70 to 100% of sprouting rate (using 50 g L^{-1} sucrose). However, Ishigaki et al. (2009) observed that treatments containing 2.0 mg L^{-1} BAP (associated with concentrations of 2,4-dichlorophenoxyacetic (2,4-D) and gibberilines) and 2 mg L^{-1} BAP associated with concentrations of naphthalene acetic acid (NAA) were efficient in embryogenic regeneration, growth and maturity of *Brachiaria ruziziensis*.

Neibaur et al. (2008) using calluses from inflorescences of *Paspalum vaginatum* Swartz recommended the propagation protocols with 1 mg L^{-1} BAP associated with 2,4-D or Dicamba and 20 g L^{-1} sucrose. Thus, they verified successes in embryogenic regenerations and future genetic transformations in this species. Similarly, Sompornpailin and Khunchuay (2016) were successful in regenerating calluses from roots in "vertiver grass" (*Vetiveria zizanioides* (L.) Nash) between 85% to 90% using the average concentration of 2.0 mg L^{-1} and 20 g L^{-1} sucrose associated with another phytoregulator.

Silva et al. (2017) tested lower concentrations of BAP (0.0; 0.25; 0.5; 1.0; 1.5 mg L^{-1}) in MS medium plus of 30 g L^{-1} sucrose and verify that despite these concentrations not having been significantly influenced, provided higher survival rate and shoots in the nodal segments of *Rosa* sp.

According to Pereira and Fortes (2003), the solidification of the medium may favor the emission of shoots depending on the species being worked on. However, these authors found gains in the efficiency of *in vitro* multiplication of the potato when the liquid culture medium was used. In the micropropagation of bromeliads (*Neoregelia cruenta*, *Tillandsia stricta*, *Vriesea gigantea*, *V. guttata* and *V. incurvata*), Mengarda et al. (2009) observed that the static liquid culture medium showed better results when compared to semi-solid and liquid under agitation media.

In the rooting phase, 144 explants originated from the sprouts obtained in the BAP and sucrose experiments were used. This enabled the rooting rate to be 45%. However, there is no information in the literature on the *in vitro* rooting rate of the species, therefore, it is not possible to say whether this value is considered low. On the other hand, it is hoped that the composition of the medium may have an effect on the rooting of canarian sprouts produced *in vitro*, therefore, it is suggested that in future studies other compositions of the medium be evaluated at this stage, for example, concentrations of sucrose and PGR. The concentration of sucrose can affect the percentage of oxidized explants due to changes in the osmotic potential of the culture medium, thus also rooting. High concentrations of salts can also be harmful, inhibiting the initial rooting due to the low nitrogen requirement for the formation of the root system (Fossard et al. 1978; Lemos et al. 2002; Thorpe et al. 2008).

The sucrose content of the culture medium in higher concentrations, in creeping river grass and other plant species such as bromeliads, potatoes and sugar cane, not only impaired the growth of the aerial part due to the osmotic potential, but also by oxidation of the medium and reduction rooting. The sucrose contents for the culture medium should be recommended intrinsically linked to the physiological state of the explant. In this way, the suggested values for creeping river grass can be adjusted during the successive subcultures. In addition, it was found that sucrose levels, when combined with other growth promoters, such as exogenous cytokinin (BAP), can be harmful, presenting a phytotoxic effect due to its excess shortening internodes and associated with excess salts can interfere in the nutrient absorption (Lane 1979; Leshem et al. 1988; Lemos et al. 2002; Rubin et al. 2007; Thorpe et al. 2008; Alcantara et al. 2014; Lemes et al. 2016).

In the present study, the reduction (30%) in the concentration of the solidifying agent did not affect the sprouting rate of the explants. Bearing in mind that the environment must have favorable conditions to support the explant and absorption of several nutritious substances (Jaeger et al. 2015).

For rooting purposes, culture media with high concentrations of salts can be harmful, inhibiting initial rooting due to the low nitrogen requirement for the formation of the root system, and therefore must be replaced by media with low concentrations of sucrose and salts (Fossard et al. 1978; Lemos et al. 2002; Thorpe et al. 2008).

5. Conclusions

Absence of contamination in the *in vitro* establishment occurred at concentrations 15, 30 and 60 g L^{-1} sucrose. The combination of 1.5 mg L^{-1} BAP and 30 g L^{-1} sucrose promoted greater induction of sprouts. In addition, the *in vitro* rooting of *E. polystachya* was 45%.

Authors' Contributions: SILVEIRA, E.M.: conception and design, acquisition of data, analysis and interpretation of data, critical review of important intellectual content, final approval; LOPES, R.: analysis and interpretation of data, critical review of important intellectual content, final approval; RODRIGUES, F.A.: analysis and interpretation of data, drafting the article, critical review of important intellectual content, edited the manuscript, final approval; RAIZER, M.D.M.: analysis and interpretation of data, critical review of important intellectual content, final approval; DIAS, F.J.: conception and design, critical review of important intellectual content, final approval; HARADA, P.K.: conception and design, critical review of important intellectual content, final approval; ARAÚJO, D.S.: conception and design, critical review of important intellectual content, final approval; PASQUAL, M.: critical review of important intellectual content, final approval; LUZ, J.M.Q.: conception and design, critical review of important intellectual content, final approval; LOPES, M.T.G.: conception and design, acquisition of data, analysis and interpretation of data, critical review of important intellectual content, final approval. All authors have read and approved the final version of the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

Ethics Approval: Not applicable.

Acknowledgments: The authors would like to thank EMBRAPA Western Amazon for the availability of infrastructure to carry out the experiments and the employees of the Plant Tissue Culture Laboratory.

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Received: 17 April 2021 | **Accepted:** 19 October 2021 | **Published:** 9 September 2022