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IN VITRO DIRECT ORGANOGENESIS FROM MICROCUTTINGS OF PLEROMA HETEROMALLUM: CONTRIBUTIONS TO THE PROPAGATION AND CONSERVATION OF A NATIVE BRAZILIAN SPECIES

ORGANOGÊNESE DIRETA IN VITRO A PARTIR DE MICROESTACAS DE PLEROMA HETEROMALLUM: CONTRIBUIÇÕES PARA A PROPAGAÇÃO E CONSERVAÇÃO DE UMA ESPÉCIE NATIVA BRASILEIRA

ORGANOGÉNESIS DIRECTA IN VITRO A PARTIR DE MICROESTACAS DE PLEROMA HETEROMALLUM: CONTRIBUCIONES A LA PROPAGACIÓN Y CONSERVACIÓN DE UNA ESPECIE NATIVA BRASILEÑA

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ABSTRACT

Pleroma heteromallum (D. Don) D. Don is an endemic species of Brazil with high ornamental potential. Seed propagation is limited due to the difficulty of embryo establishment, resulting from the reduced or even absent endosperm, which makes stem cuttings the conventional propagation method. In this context, micropropagation emerges as a viable alternative for large-scale seedling production, with phytosanitary quality and year-round availability. This study aimed to evaluate the morphogenic response of P. heteromallum microcuttings cultivated in vitro and their performance during acclimatization. Four in vitro experiments were carried out using MS medium with different combinations of BAP, IBA, PVP, and activated charcoal, aiming at shoot and root induction. The experiment followed a completely randomized design, under controlled conditions of 25±1°C, irradiance of 25 µmol m^{-2} s^{-1}, and a 16-hour photoperiod. The parameters evaluated were microcutting survival, explant oxidation, number of leaves, shoot length, length of the longest root, number of roots, and number of nodes. The plantlets obtained were transferred to pots with commercial substrate and acclimatized in a greenhouse for 30 days. The antioxidant PVP showed an adverse effect, promoting explant oxidation, while IBA inhibited root elongation. The best development was observed in MS medium with 100% salt concentration and free of plant growth regulators, offering greater efficiency and lower cost. During acclimatization, the survival rate was 67%. Direct organogenesis-based micropropagation is therefore a promising strategy for the production of P. heteromallum seedlings, contributing to its conservation and ornamental use.

Keywords: Jaguar's Ear. In Vitro Propagation. Micropropagation. Acclimatization. Seedling Production.

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Revista ARACÊ
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RESUMO

Pleroma heteromallum (D. Don) D. Don é uma espécie endêmica do Brasil com elevado potencial ornamental. A propagação por sementes é limitada devido ao comprometimento do desenvolvimento embrionário, causado pela redução ou até ausência do endosperma, por isso, a propagação por estacas é comumente utilizada como método convencional. Nesse contexto, a micropropagação surge como uma alternativa viável para a produção de mudas em larga escala, com qualidade fitossanitária e disponibilidade durante todo o ano. Este estudo teve como objetivo avaliar a resposta morfogênica de microestacas de P. heteromallum cultivadas in vitro e seu desempenho durante a aclimatização. Quatro experimentos in vitro foram conduzidos utilizando o meio MS com diferentes combinações de BAP, AIB, PVP e carvão ativado, visando à indução de brotos e raízes. O experimento seguiu um delineamento inteiramente casualizado, sob condições controladas de 25±1°C, irradiância de 25 µmol m^{-2} s^{-1} e fotoperíodo de 16 horas. Os parâmetros avaliados foram: sobrevivência das microestacas, oxidação dos explantes, número de folhas, comprimento dos brotos, comprimento da maior raiz, número de raízes e número de nós. As plântulas obtidas foram transferidas para vasos contendo substrato comercial e aclimatizadas em casa de vegetação por 30 dias. O antioxidante PVP apresentou efeito adverso, promovendo oxidação dos explantes, enquanto o AIB inibiu o alongamento radicular. O melhor desenvolvimento foi observado no meio MS com 100% da concentração de sais e isento de reguladores de crescimento, proporcionando maior eficiência e menor custo. Durante a aclimatização, a taxa de sobrevivência foi de 67%. Assim, a micropropagação baseada em organogênese direta mostra-se uma estratégia promissora para a produção de mudas de P. heteromallum, contribuindo para sua conservação e uso ornamental.

Palavras-chave: Orelha-de-onça. Propagação in Vitro. Micropropagação. Aclimatização. Produção de Mudas.

RESUMEN

Pleroma heteromallum (D. Don) D. Don es una especie endémica de Brasil con alto potencial ornamental. La propagación por semillas es limitada debido al compromiso del desarrollo embrionario, causado por la reducción o incluso ausencia del endospermo, por esta razón, la propagación por estacas se utiliza comúnmente como método convencional. En este contexto, la micropropagación surge como una alternativa viable para la producción de plántulas a gran escala, con calidad fitosanitaria y disponibilidad durante todo el año. Este estudio tuvo como objetivo evaluar la respuesta morfogénica de microestacas de P. heteromallum cultivadas in vitro y su desempeño durante la aclimatación. Se realizaron cuatro experimentos in vitro utilizando el medio MS con diferentes combinaciones de BAP, AIB, PVP y carbón activado, con el propósito de inducir brotes y raíces. El experimento siguió un diseño completamente al azar, bajo condiciones controladas de 25±1°C, irradiancia de 25 µmol m^{-2} s^{-1} y fotoperíodo de 16 horas. Los parámetros evaluados fueron: supervivencia de las microestacas, oxidación de los explantes, número de hojas, longitud de los brotes, longitud de la raíz más largas, número de raíces y número de nudos. Las plántulas obtenidas fueron transferidas a macetas con sustrato comercial y aclimatadas en invernadero durante 30 días. El antioxidante PVP mostró un efecto adverso, promoviendo la oxidación de los explantes, mientras que el AIB inhibió el alargamiento radicular. El mejor desarrollo se observó en el medio MS con 100% de concentración de sales y sin reguladores de crecimiento, proporcionando mayor eficiencia y menor costo. Durante la aclimatación, la tasa de supervivencia fue del 67%. Por lo tanto, la micropropagación basada en organogénesis

Revista ARACÊ
ISSN: 2358-2472

directa se presenta como una estrategia prometedora para la producción de plántulas de P. heteromallum, contribuyendo a su conservación y uso ornamental.

Palabras clave: Orelha-de-onça. Propagación in Vitro. Micropropagación. Aclimatación. Producción De Plántulas.

Revista ARACÊ
ISSN: 2358-2472

1 INTRODUCTION

The family Melastomataceae comprises 177 genera and approximately 5,750 species. In Brazil, it includes around 69 genera and 1.430-1.440 species, occurring across all phytogeographic domains, with particular prominence in the Atlantic Forest, Cerrado, and Amazon regions (Goldenberg et al. 2020). Species of this family are relevant for ecosystem restoration, avian feeding, pharmaceutical production, fencing, tool handles, posts, and ornamental purposes (Fernández-Sánchez et al. 2020; Nauli et al. 2020; Burgos et al. 2023).

Pleroma heteromallum (D.Don) D.Don, commonly known as jaguar's ear, is a perennial shrub with purple flowers and hairy, cordate leaves, exhibiting ornamental, phytoremediative, and medicinal potential (Kuster et al. 2009; Lorenzi 2015; Cuevas-Reyes et al. 2018). Species of the genus *Pleroma*, known as "quaresmeiras," show difficulties in seed propagation due to low germination rates and handling of small seeds, and are commonly propagated vegetatively through cuttings or air-layering (Lorenzi 2015; Fragoso et al. 2017; Latoh et al. 2022). However, traditional vegetative propagation is limited by seasonality, high material demand, and seedling health constraints (Silva et al. 2019; Latoh et al. 2022).

In vitro micropropagation emerges as an efficient alternative, enabling large-scale production, phytosanitary quality, and independence from seasonal constraints (Albino et al. 2019). This technique involves the cultivation of explants in culture medium under aseptic and controlled conditions (Hasnain et al. 2022). Acclimatization represents the final, critical stage for the adaptation of plantlets to ex vitro conditions, often accounting for the greatest losses in *in vitro* plant cultivation (Rodrigues et al. 2019; Vicente; Araujo 2020).

Direct organogenesis, based on organ formation from meristems, is a common and efficient method for micropropagation (Hasnain et al. 2022). Among the advantages of micropropagation are the possibility of production in small areas and at any time of the year, high phytosanitary quality, support for genetic studies, germplasm banking, and preservation of genetic variability (Pêgo et al. 2015; Prudente et al. 2016; Rodrigues et al. 2019; Plessis et al. 2020).

Despite the various potential uses of *P. heteromallum*, research on its micropropagation remains scarce. In this context, developing an efficient protocol for *in vitro* cultivation represents a promising strategy to expand seedling production, meeting both commercial demands and species conservation needs. Therefore, the present study aimed

Revista ARACÊ
ISSN: 2358-2472

to evaluate the morphogenic response of *P. heteromallum* microcuttings cultivated *in vitro* and their performance during the acclimatization phase.

2. MATERIAL AND METHOD

2.1 IN VITRO ESTABLISHMENT OF *Pleroma heteromallum* MICROCUTTINGS

Microcuttings approximately 1.5 cm in length, obtained from *in vitro* germinated *P. heteromallum* seedlings, were inoculated into test tubes containing MS (Murashige; Skoog 1962) culture medium at full salt strength, supplemented with 30 g L^{-1} sucrose and 7.0 g L^{-1} agar, constituting the control treatment (T1). The other treatments consisted of the same basal medium supplemented with: 4.9 µM indole-3-butyric acid (IBA), 4.4 µM 6-benzylaminopurine (BAP), and 1 g L^{-1} polyvinylpyrrolidone (PVP) (T2); 4.9 µM IBA, 4.4 µM BAP, and 1 g L^{-1} activated charcoal (T3); 4.9 µM IBA and 4.4 µM BAP (T4); and 4.4 µM BAP (T5). The pH of the media was adjusted to 5.8 ± 0.1 before agar addition. Sterilization was performed in an autoclave at 121 °C and 1.2 atm for 20 minutes.

The experimental design was completely randomized (CRD), with seven replicates, each experimental unit consisting of a tube containing approximately 10 mL of culture medium and a single microcutting. Cultures were maintained in a B.O.D. growth chamber at 25 ± 1 °C, with a 16-hour photoperiod and irradiance of approximately 25 µmol m^{-2} s^{-1} provided by white fluorescent lamps.

After 42 days of cultivation, the following parameters were evaluated: survival percentage (SP), oxidation percentage (OP), callogenesis percentage (CP), shoot percentage (ShP), rooting percentage (RP), number of shoots (NS), number of roots (NR), length of the longest shoot (LLS) and length of the longest root (LLR).

Data were tested for normality using the Shapiro–Wilk test (5%). When necessary, data were transformed using $[(x + 1)^{0.5}]$. Analysis of variance (ANOVA) was performed, and when significant, means were compared using Tukey's test at 5% probability. All analyses were conducted using the SISVAR 5.8 *software* (Ferreira 2011).

2.2 IN VITRO SUBCULTURE OF *Pleroma heteromallum* SHOOTS

Explants bearing shoots from treatments T1 and T3 of the previous experiment were transferred to 200 mL glass jars containing MS medium at full salt strength, supplemented with 30 g L^{-1} sucrose and 7.0 g L^{-1} agar. The pH was adjusted to 5.8 ± 0.1 prior to sterilization in an autoclave (121 °C for 20 minutes).

Revista ARACÊ
ISSN: 2358-2472

The experimental design was completely randomized (CRD), with seven replicates, each experimental unit consisting of a 200 mL jar containing 20 mL of medium and a single explant. Environmental conditions were the same as in the previous experiment: 25 ± 1 °C, 16-hour photoperiod, and irradiance of 25 μmol m^{-2} s^{-1}.

After 35 days of *in vitro* cultivation, the following parameters were evaluated: survival percentage (SP), the number of shoots (NS), the length of the largest shoot (LLS), the number of leaves of the largest shoot (NLLS) and the number of root (NR) of the seedlings. Data were subjected to the same statistical procedures described for the previous experiment.

2.3 IN VITRO RHIZOGENESIS OF *Pleroma heteromallum* SHOOTS

Shoots approximately 3.0 cm in length, obtained from the previous subculture, were inoculated in MS medium containing 0.0 μM (control – T1), 2.5 μM (T2), 4.9 μM (T3), 7.4 μM (T4), and 9.8 μM (T5) of IBA, supplemented with 30 g L^{-1} sucrose and 7.0 g L^{-1} agar. The pH was adjusted to 5.8 ± 0.1 prior to autoclaving at 121 °C for 20 minutes.

The experiment was conducted in a completely randomized design (CRD), with five treatments and eight replicates. Each experimental unit consisted of a glass tube containing approximately 20 mL of culture medium and a single shoot. The environmental growth conditions were the same as in the previous experiments.

After 42 days of *in vitro* cultivation, the following parameters were evaluated: survival percentage (SP); oxidation percentage (OP); number of leaves (NL); shoot length (SL); length of the longest root (LLR); number of root (NR); superficial root number (SRN); node number (NN).

The data were tested for normality using the Shapiro–Wilk test (5%) and, when necessary, transformed [$(x + 1)^{0.5}$]. ANOVA was performed at 5% probability, and when significant, regression analysis was applied. Statistical analyses were conducted using SISVAR 5.8 (Ferreira 2011), and regression graphs were constructed in Microsoft Excel® 2010.

Additionally, a Principal Component Analysis (PCA) was performed to identify grouping patterns of the response variables according to treatments. The treatment means were previously standardized. The similarity matrix was constructed based on Pearson's correlation (1901), and the analysis was performed using the R software (R Core Team 2021).

Revista ARACÊ
ISSN: 2358-2472

2.4 IN VITRO ROOT ELONGATION AND DEVELOPMENT OF *Pleroma heteromallum*

Aiming at root elongation (positive gravitropism) of the plantlets obtained from the rooting experiment, which had shown inadequate root development, a 60-day subculture was performed in MS medium (full-strength salts) supplemented with 30 g L^{-1} sucrose and 7.0 g L^{-1} agar. The pH was adjusted to 5.8 ± 0.1, and the medium was sterilized by autoclaving at 121 °C and 1.2 atm for 20 minutes.

The experiment was carried out in a completely randomized design (CRD), considering the previous IBA treatments (T1 – 0.0 µM; T2 – 2.5 µM; T3 – 4.9 µM; T4 – 7.4 µM; T5 – 9.8 µM), with four replicates and two plantlets per flask, totaling 20 observation units. The flasks (200 mL) were sealed with transparent plastic caps covered with PVC film and maintained under the same environmental conditions as in the previous experiments.

After 60 days of subculture, the plantlets were removed from the flasks and their roots were washed in running water until the culture medium was completely eliminated. The following parameters were evaluated: total fresh mass (MF), shoot length (SL), number of leaves (NL), length of the largest leaf (LLL), number of roots (NR), length of the longest root (LLR) and number of nodes (NN) of the seedlings.

Total fresh mass was determined using a digital precision balance (four decimal places). Measurements of SL, LLL, and LLR were taken with a millimeter ruler, while NL, NR, and NN were obtained by direct counting.

The data were tested for normality using the Shapiro–Wilk test at 5% probability. For significant variables, data were transformed according to the equation $[(x + 1)^{0.5}]$. Subsequently, analysis of variance (ANOVA) was performed at 5% probability, and when significant, means were compared using Tukey's test at 5% probability. Statistical analyses were performed using SISVAR 5.8 (Ferreira 2011).

2.5 ACCLIMATIZATION OF PLANTLETS

For the acclimatization stage, plantlets from the previous experiment were transferred to polyethylene pots (n°6 – 80 mL) containing commercial substrate and subsequently acclimatized in a greenhouse covered with translucent polypropylene tiles. The experiment was conducted in a completely randomized design (CRD), with 30 plantlets, each pot representing one experimental unit. Irrigation was performed manually every two days, with the application of 70 mL of water per pot. After 30 days, the survival percentage of plantlets was evaluated.

Revista ARACÊ
ISSN: 2358-2472

3 RESULTS

3.1 IN VITRO ESTABLISHMENT OF *Pleroma heteromallum* MICROCUTTINGS

The normality test was significant for all variables; therefore, data were transformed prior to performing the analysis of variance, which was also significant for all variables analyzed. The results of the mean comparison test for the vegetative propagation of *P. heteromallum* via microcuttings are presented in Table 1.

Table 1

Means of survival percentage (SP), oxidation percentage (OP), callogenesis percentage (CP), shoot percentage (ShP), rooting percentage (RP), number of shoots (NS), number of roots (NR), length of the largest shoot (LLS) and length of the largest root (LLR) of P. heteromallum microcuttings after 42 days of inoculation in different culture media

Treatment	SP	OP	CP	ShP	RP	NS	NR	LLS	LLR
	%					un		cm	
T1 (Control)	100.00 a	0.00a	0.00a	100.00 a	85.71a	5.43a	2.43 b	1.73a b	0.71a b
T2 (IBA + BAP + PVP)	28.57b	71.43b	14.28 b	42.86b	0.00b	1.43b	0.00 b	0.26b	0.00b
T3 (IBA + BAP + AC)	100.00 a	0.00a	0.00a	100.00 a	100.00 a	3.43a b	6.71 a	2.50a	1.29a
T4 (IBA + BAP)	28.57b	71.43b	42.86c	28.57b	28.57b	1.14b	1.57 b	1.24a b	0.41b
T5 (BAP)	71.43a b	28.57a b	0.00a	100.00 a	14.28b	2.43a b	0.14 b	0.93a b	0.11b
Overall Mean	65.71	34.29	11.43	74.29	45.71	2.77	2.17	1.33	0.51
CV (%)	12.31	13.71	11.58	10.25	11.27	37.53	35.64	24.56	17.29

Note: Means followed by the same letter in the column do not differ from each other according to Tukey's test at 5% probability; ns: not significant by F test at 5%; CV: coefficient of variation. Legend: MS medium (T1 – control); MS medium supplemented with 4.9 µM IBA, 4.4 µM BAP, and 1 g L^{-1} PVP (T2); MS medium supplemented with 4.9 µM IBA, 4.4 µM BAP, and 1 g L^{-1} activated charcoal (T3); MS medium supplemented with 4.9 µM IBA and 4.4 µM BAP (T4); and MS medium supplemented with 4.4 µM BAP (T5). Source: elaborated by the authors.

It can be observed that no oxidation occurred in T1 (control) and T3 (medium supplemented with activated charcoal), and the survival rate of these treatments was statistically higher than the others. Treatments supplemented with PVP (T2) and with BAP and IBA (T4) in the culture medium showed approximately 71% of oxidized explants. In the

Revista ARACÊ
ISSN: 2358-2472

treatment supplemented with IBA (T5), 28.57% of explants were oxidized, which did not differ statistically from any of the other treatments.

Callus formation, which was undesired in this experiment, was observed in treatments T2 and T4, with 14.28% and 42.86% of explants forming callus, respectively. Depending on the objective of the *in vitro* culture, these treatments could be interesting, for example, for plant regeneration via indirect organogenesis.

One hundred percent of explants developed shoots in T1, T3, and T5, and these treatments were statistically superior to T2 (42.86%) and T4 (28.57%), which did not differ statistically from each other. However, subsequently in T5, approximately 29% of the explants became oxidized.

Regarding the number of shoots, T1 was statistically superior to the other treatments, with a mean of 5.43 shoots per microcutting.

In T3, 100% of the explants developed roots, whereas in T1, T2, T4, and T5 this percentage was 85.71%, 0%, 28.57%, and 14.28%, respectively. Statistically, T1 and T3 were superior to the other treatments in terms of rooting percentage, and both did not differ from each other. Regarding the number of roots, T3 was statistically superior to the other treatments, with a mean of 6.71 roots per microcutting.

The results suggest a negative or null effect of BAP addition on shoot induction and number of shoots, and a beneficial effect of IBA supplementation on root induction and number of roots in P. heteromallum microcuttings cultured in vitro. T3 was significantly superior for the variables length of the longest shoot and length of the longest root, indicating a positive effect of the combination of IBA, BAP, and activated charcoal on these parameters.

For *in vitro* vegetative propagation of *P. heteromallum* from microcuttings, it can be noted that the antioxidant PVP had an antagonistic effect, as explant oxidation occurred in this treatment. In the treatment with activated charcoal, no oxidation or callus formation was observed, suggesting that activated charcoal may inhibit this process.

Treatments T1 and T3 proved to be the most promising for obtaining shoots and rooting from *P. heteromallum* microcuttings cultured in vitro. Therefore, producers should consider the cost-benefit ratio when choosing between these treatments.

Revista ARACÊ
ISSN: 2358-2472

3.2 IN VITRO SUBCULTURE OF *Pleroma heteromallum* SHOOTS

The normality test was significant for the variables number of shots (NS) and number of leaves of the largest shoot (NLLS), so data were transformed prior to performing the analysis of variance.

According to the analysis of variance, no significant differences were observed between treatments T1 and T3 for the evaluated traits (Table 2) during the subculture stage of the plantlets in MS medium with 100% of the original salt concentration, free of plant growth regulators and antioxidants. One hundred percent survival of the plantlets was obtained in both treatments.

Table 2

Means of explant survival percentage (SP), number of shoots (NS), number of roots (NR), number of leaves of the largest shoot (NLLS) and length of the largest shoot (LLS) after 35 days of in vitro subculture of P. heteromallum plantlets in MS medium with 100% of the original salt concentration, free of plant growth regulators

Treatment	SP	NS	NR	NLLS	LLS
	%		un		cm
T1 (Control)	100.00 ns	6.71 ns	5.86 ns	8.14 ns	4.01 ns
T3 (IBA + BAP + AC)	100.00 ns	3.86 ns	7.00 ns	8.29 ns	5.20 ns
Overall Mean	100.00	5.29	6.43	8.21	4.61
CV (%)	0.00	31.02	26.36	8.83	16.03

Note: ns: not significant by F test ($P \leq 0.05$); CV: coefficient of variation. Legend: plantlets derived from *in vitro* culture in MS medium with 100% of the original salt concentration (T1); plantlets derived from *in vitro* culture in MS medium with 100% of the original salt concentration supplemented with 4.9 µM IBA, 4.4 µM BAP, and 1 g L^{-1} activated charcoal (T3). Source: elaborated by the authors.

3.3 IN VITRO RHIZOGENESIS OF *Pleroma heteromallum* SHOOTS

The normality test was significant for all evaluated variables, so data were transformed prior to performing the analysis of variance. Mean results from the *in vitro* rooting stage of *P. heteromallum* shoots are presented in Table 3.

Revista ARACÊ
ISSN: 2358-2472

Table 3

Mean values of survival percentage (SP), oxidation percentage (OP), number of leaf (NL), number of roots (NR), superficial root number (SRN), node number (NN), shoot length (SL) and length of the longest root (LLR) in P. heteromallum shoots after 60 days of inoculation in different in vitro rooting media

Treatment	SP	OP	NL	NR	SRN	NN	SL	LLR
	%		un				cm	
T1 (control)	100.00	0.00	17.38	6.63	4.75	5.00	4.98	3.63
T2 (2.5 µM de IBA)	50.00	50.00	6.50	0.89	3.50	2.75	2.46	0.11
T3 (4.9 µM de IBA)	50.00	50.00	8.25	0.00	4.25	2.00	2.39	0.00
T4 (7.4 µM de IBA)	100.00	0.00	23.38	0.00	16.50	4.00	4.51	0.00
T5 (9.8 µM de IBA)	62.50	37.50	8.88	0.00	7.38	3.38	4.81	0.00
Overall Mean	72.50	27.50	12.88	1.50	7.28	3.43	3.83	0.75

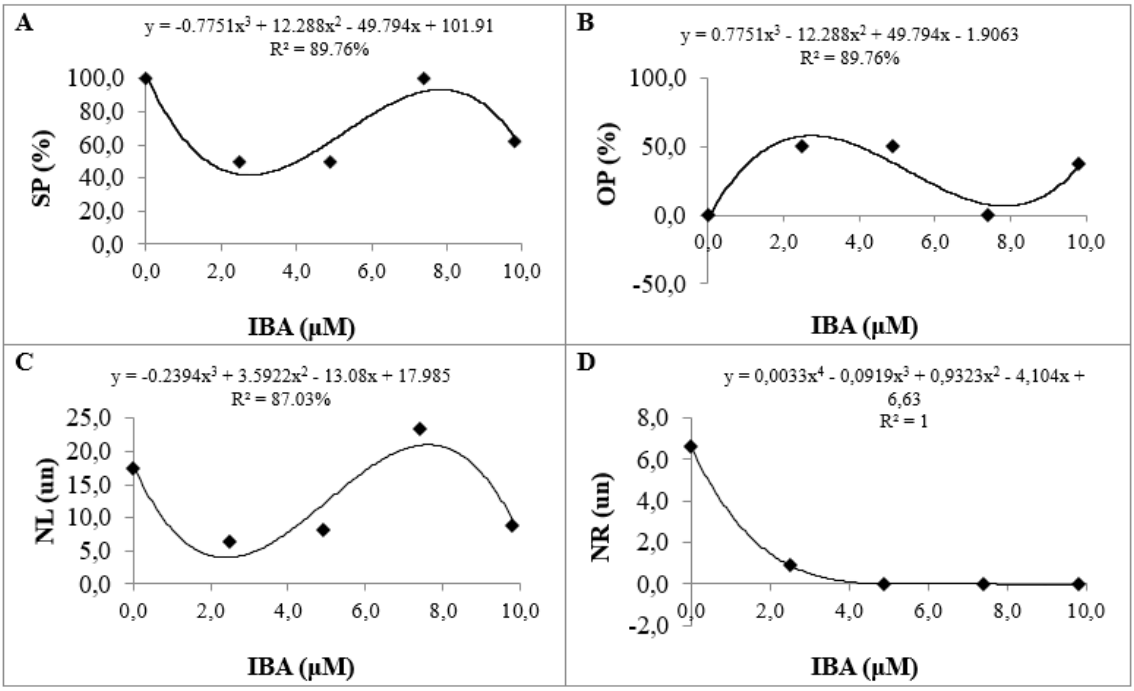
Source: elaborated by the authors.

According to the analysis of variance, no significant differences were observed for shoot length and number of nodes of the sprouted microcuttings. Figure 1 shows the regression analysis for the variables survival percentage (SP), oxidation percentagem (OP), number of leaf (NL) and number of roots (NR) in relation to the different IBA concentrations tested.

Revista ARACÊ
ISSN: 2358-2472

Figure 1

Polynomial regression of mean values for survival percentage (A), oxidation percentage (B), number of leaves (C) and number of roots (D) in Pleroma heteromallum microcuttings cultured in vitro for 42 days in MS medium containing different IBA concentrations.



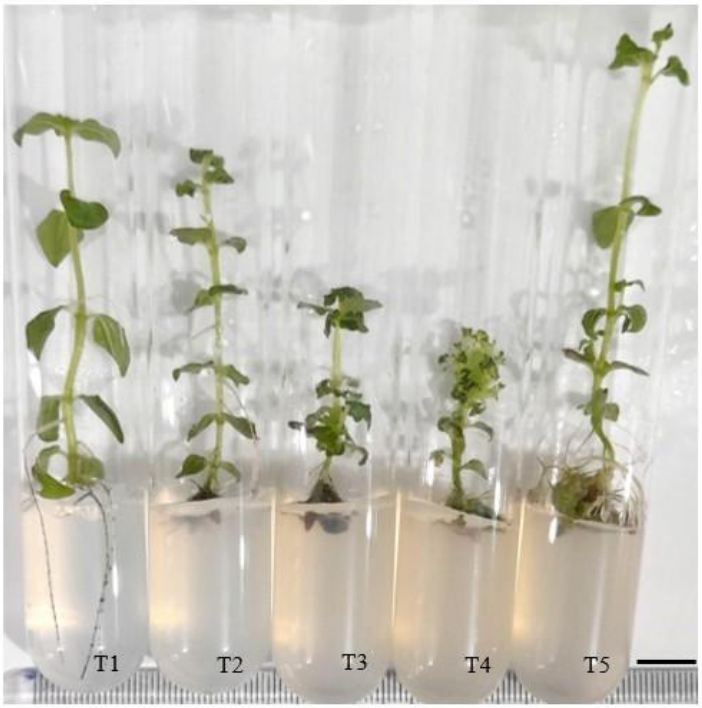
Source: elaborated by the authors.

The highest survival percentage of the microcuttings (100%) was observed both in the absence of IBA in the culture medium (T1) and in the treatment with 7.4 µM IBA (T4) (Figure 1A). In these same treatments, the oxidation rate was null (0%) (Figure 1B). The highest number of leaves was recorded in the treatment with 7.4 µM IBA (T4) (Figure 1C), whereas the total number of roots was higher in the MS medium without IBA (T1) (Figure 1D). However, in T4, although the plantlets showed the highest mean number of leaves, these leaves were smaller in size and accompanied by shortened internodes, typical characteristics of hyperhydricity. These findings suggest that, despite promoting leaf production, the higher IBA concentration may compromise the normal morphology of the shoots. The morphological aspects of the plantlets in the different treatments are shown in figure 2, allowing a comparative visualization of these effects.

Revista ARACÊ
ISSN: 2358-2472

Figure 2

Pleroma heteromallum shoots in different rooting media after 42 days of in vitro culture.



Legend: MS medium supplemented with 0.0 µM IBA – control (T1); MS medium supplemented with 2.5 µM IBA (T2); MS medium supplemented with 4.9 µM IBA (T3); MS medium supplemented with 7.4 µM IBA (T4); and MS medium supplemented with 9.8 µM IBA (T5). Scale bar = 1.0 cm. Source: elaborated by the authors.

Moreover, the number of superficiais roots, those that did not grow toward the medium, was also higher in T4, although with reduced length. On the other hand, the length of the longest root, i.e., those that developed toward the medium, was greater in T1, suggesting that the presence of IBA negatively affects root elongation. It should be noted that in T4 the shoots exhibited hyperhydricity, an undesired anomaly in in vitro plant culture.

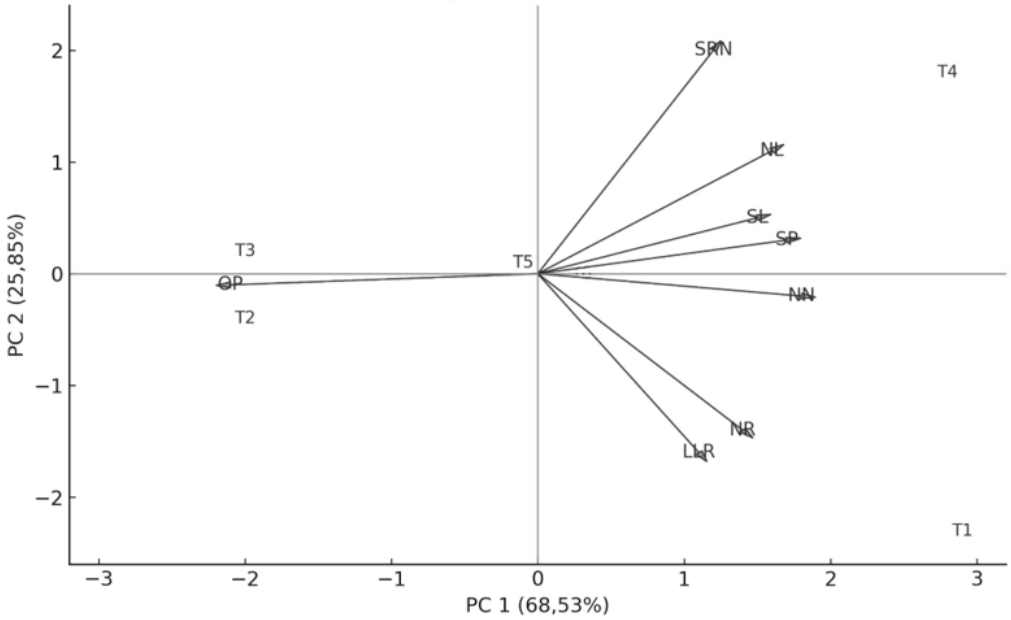
Results from the Principal Component Analysis (PCA) indicated no multicollinearity issues and showed that the first two components explained 94.38% of the variability among the applied treatments and analyzed variables, with the first principal component accounting for 68.53% and the second for 25.85% of this variability.

The scatter plot analysis (Figure 3) demonstrated that T2 and T3 showed a positive correlation with shoot oxidation rate. Meanwhile, T1 (control) displayed a positive correlation with the number of roots and length of the longest root, and T4 showed a positive correlation with the number of superficiais roots.

Revista ARACÊ
ISSN: 2358-2472

Figure 3

Principal Component Analysis (PCA) plot showing the relationship between treatments (culture media) and variables evaluated during in vitro rooting of Pleroma heteromallum shoots



Note: Variable positions reflect their relationships with each other and with the treatments; closer proximity indicates higher correlation. Legend: survival percentage (SP); oxidation percentage (OP); number of leaves (LN); shoot length (SL); length of the longest root (LLR); number of root (NR); superficial root number (SRN); node number (NN); MS medium with 0.0 µM IBA – control (T1); MS + 2.5 µM IBA (T2); MS + 4.9 µM IBA (T3); MS + 7.4 µM IBA (T4); MS + 9.8 µM IBA (T5). Source: elaborated by the authors.

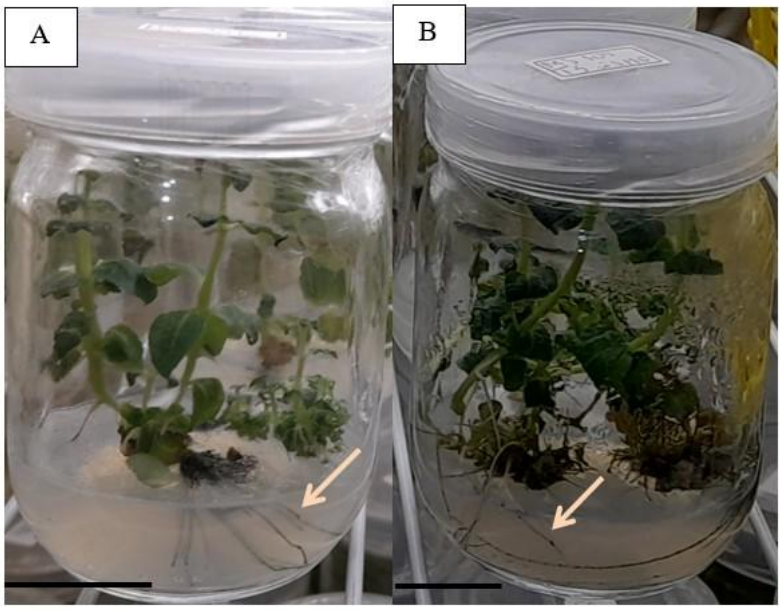
3.4 ROOT ELONGATION AND *IN VITRO* DEVELOPMENT OF *Pleroma heteromallum*

After 60 days of subculture of the seedlings, growth of the aerial part, including leaves and stem, as well as roots growth toward the MS medium without plant growth regulators and antioxidants, was observed (Figure 4). This process did not occur in P. heteromallum shoots cultured on MS medium containing IBA, suggesting an antagonistic effect of the addition of this plant growth regulator on root development in the seedlings.

Revista ARACÊ
ISSN: 2358-2472

Figure 4

Pleroma heteromallum shoots after 60 days of subculture on MS medium with 100% of the original salt concentration and without plant growth regulators. Arrows indicate roots that elongated during the subculture of shoots from treatments T3 (A) and T5 (B)



Scale bars: 2.5 cm. Source: elaborated by the authors.

O teste de normalidade foi significativo para as variáveis LLL e LRL, de modo que as mesmas foram transformadas antes da realização da análise de variância. A análise de variância foi significativa para todas as variáveis analisadas e o resultado do teste de médias encontra-se na Tabela 4.

Table 4

Mean values of total fresh mass (FM), shoot length (SL), largest leaf length (LLL), longest root length (LRL), number of leaf (LN), number of root (NR) and node number (NN) in P. heteromallum shoots after 60 days of subculture on MS medium with 100% of the original salt concentration, without plant growth regulators and antioxidant

Treatment	FM	SL	LLL	LRL	NL	NR	NN
	g		cm			un	
T1 (originating from control 0.0 µM de IBA)	1.17a	9.45b	1.18a	7.18a	42.50b	13.75b	10.25ab
T2 (originating from 2.5 µM de IBA)	0.45b	9.35b	1.53a	1.38b	17.25c	11.00b	8.75bc

Revista ARACÊ
ISSN: 2358-2472

T3 (originating from 4.9 µM de IBA)	0.99a b	8.18bc	1.28 a	3.78a b	30.50b c	12.75 b	10.00a b
T4 (originating from 7.4 µM de IBA)	1.78a	2.53c	0,01 b	1.95b	69.75a	10.00 b	2.75c
T5 (originating from 9.8 µM de IBA)	1.70a	18.50 a	1.60 a	3.38a b	35.50b c	24.75 a	16.75a
Overall mean	1.22	9.60	1.12	3.53	39.10	14.45	9.70
CV (%)	37.09	31.53	6.28	22.55	21.61	34.66	33.78

Note: CV: coefficient of variation; means followed by the same letter in the column do not differ significantly according to Tukey's test at 5% probability. Legend: Seedlings originating from *in vitro* culture on MS medium supplemented with 0.0 µM IBA – control (T1); seedlings originating from *in vitro* culture on MS medium supplemented with 2.5 µM IBA (T2); seedlings originating from *in vitro* culture on MS medium supplemented with 4.9 µM IBA (T3); seedlings originating from *in vitro* culture on MS medium supplemented with 7.4 µM IBA (T4); seedlings originating from *in vitro* culture on MS medium supplemented with 9.8 µM IBA (T5). Source: elaborated by the authors.

Seedlings originating from treatments T4 and T5 showed the highest mean values for the variable FM. The mean comparison test indicated that seedlings from T5 had statistically higher means for SL, RN and NN compared to the other treatments. Regarding LLL, T4 was lower than the other treatments, which did not differ significantly among themselves. For LRL, T1, T3 and T5 showed statistically higher means than the remaining treatments. However, T3 and T5 did not differ from T2. Meanwhile, T4 was statistically lower than all other treatments for this variable.

3.5 ACCLIMATIZATION OF SEEDLINGS

After 30 days of acclimatization, a survival rate of 67% was observed for *P. heteromallum* seedlings. Seedlings originating from treatment T4 still showed signs of hyperhydricity, whereas those from treatments T1, T2, and T5 exhibited greater vigor at the end of the period (Figure 5). These results highlight the importance of the in vitro stage in determining the final quality of the seedlings, demonstrating that the previous culture conditions and treatments directly influence acclimatization success.

Revista ARACÊ
ISSN: 2358-2472

Figure 5

Pleroma heteromallum seedlings after 30 days of acclimatization in Multiplant® substrate

Legend: Seedlings originating from *in vitro* culture on MS medium supplemented with 0.0 µM IBA – control (T1); seedlings originating from *in vitro* culture on MS medium supplemented with 2.5 µM IBA (T2); seedlings originating from *in vitro* culture on MS medium supplemented with 4.9 µM IBA (T3); seedlings originating from *in vitro* culture on MS medium supplemented with 7.4 µM IBA (T4); seedlings originating from *in vitro* culture on MS medium supplemented with 9.8 µM IBA (T5). Scale bar: 1 cm. Source: elaborated by the authors.

4 DISCUSSION

It was observed that the addition of the plant growth regulators BAP and IBA to the culture medium contributed to the oxidation of *Pleroma heteromallum* microcuttings, even in the presence of the antioxidant PVP. However, the use of activated charcoal proved to be efficient, controlling 100% of oxidation. This result can be attributed to the different mechanisms of action of the antioxidants used. According to Goulart et al. (2010), activated charcoal acts by adsorbing exudates released by explants, which are responsible for oxidation. In addition, it can adsorb and reduce the availability of exogenous auxin present in the culture medium. On the other hand, PVP reacts directly with oxidizing compounds, and its main effect is related to inhibiting the release of phenolic compounds.

Revista ARACÊ
ISSN: 2358-2472

The addition of BAP and IBA to the culture medium showed an antagonistic effect on root and shoot promotion. However, in treatment T2, although these regulators were present, they were likely adsorbed by the activated charcoal, rendering them unavailable to the explants (microcuttings). Therefore, no antagonistic effect of these regulators was observed. Similarly and Puttkammer (2015) reported that IBA negatively affected the number of shoots and roots when not combined with activated charcoal during strawberry micropropagation.

The treatment consisting of BAP + IBA + activated charcoal showed superiority regarding root number, largest shoot length, and longest root length. Kozak and Wnuk (2012) observed that the combined application of BAP and IAA (indole-3-acetic acid) had no significant effect on the induction of axillary shoots of *Tibouchina urvilleana* (Melastomataceae), and also noted that shoot length was inhibited with increasing BAP concentration in the culture medium.

However, when considering the cost-benefit relationship of adding growth regulators and antioxidants to the culture medium, the treatment without these compounds (control) can be considered the most suitable for establishing *P. heteromallum* microcuttings *in vitro*. Flores et al. (2015) also suggest using MS medium without growth regulators to optimize in vitro plant production of *Ipomoea batatas* (sweet potato) cultivars.

The results of *in vitro* rhizogenesis showed that adventitious roots grew in length both in medium without the growth regulator IBA (control treatment) and at the concentration of 2.5 µM IBA. However, in the IBA-free medium, root number and longest root length were statistically higher. It was also observed that in all treatments, lateral adventitious roots, here referred to as superficiais roots because they do not grow toward the medium, were produced.

Furthermore, results from the rhizogenesis assay, conducted in the third experiment, suggest that IBA may contribute to microcutting oxidation, as no oxidation was observed in the medium without this growth regulator. Latoh et al. (2018; 2022) tested different IBA concentrations for rooting of *Tibouchina heteromalla* (syn. *P. heteromallum*) cuttings and concluded that the use of this regulator is unnecessary for adventitious root induction. These findings support the present study, suggesting that *P. heteromallum* possesses sufficient endogenous auxin concentrations, which may explain the observed antagonistic effect of IBA on *in vitro* rhizogenesis of microcuttings.

During rhizogenesis, especially in woody species, auxin toxicity may occur, which becomes evident during root elongation. Radmann et al. (2002) recommended using two

Revista ARACÊ
ISSN: 2358-2472

culture media in the rhizogenesis process: initially, the cutting is cultured in medium containing auxin to promote root induction; subsequently, it should be transferred to auxin-free medium to stimulate root growth and elongation. These studies support the results observed in the fourth experiment, in which transferring seedlings to MS medium without growth regulators resulted in root elongation toward the culture medium.

Additionally, shoots cultured in medium containing 7.4 µM IBA exhibited hyperhydricity, an undesirable condition in *in vitro* multiplication of species. Morphologically, hyperhydric plants may display turgid, thick, wrinkled, twisted, translucent, rigid, and fragile stems and leaves, shortened internodes, and an appearance indicative of excessive water accumulation (Vasconcelos et al. 2012).

Hyperhydricity is a common physiological disorder in *in vitro* plant cultures, mainly influenced by the mineral and hormonal composition of the medium, as well as culture conditions (Polivanova; Bedarev 2022). Based on this, it is suggested that the 7.4 µM IBA concentration was the determining factor for hyperhydricity in the shoots, as this disorder was not observed in the other treatments.

In this study, a 67% survival rate of *P. heteromallum* seedlings was recorded during acclimatization, higher than reported by Prudente et al. (2016), who observed approximately 55% survival of *Miconia ligustroides* seedlings (Melastomataceae) at the same stage. On the other hand, studies with other Melastomataceae species report even higher survival percentages. Zeng et al. (2008) reported 86% survival of *Tigridiopalma magnifica* seedlings after 30 days of acclimatization in a greenhouse. Similarly, Lawrence and Murugan (2017) reported 90% survival during acclimatization of *Osbeckia aspera*, while Burgos et al. (2019) obtained about 94% survival of *Bucquetia glutinosa* seedlings during this stage.

The survival rate obtained for *P. heteromallum* seedlings can be considered satisfactory. However, adjustments to the methodology or further experiments are recommended to optimize this stage and, consequently, increase the survival rate during acclimatization.

5 CONCLUSION

The results of this study demonstrate that direct organogenesis from microcuttings constitutes an effective technique for the in vitro propagation of *Pleroma heteromallum*. The use of MS medium, even in the absence of plant growth regulators, proved efficient for shoot induction and development. Furthermore, the acclimatization process in a greenhouse was

Revista ARACÊ
ISSN: 2358-2472

viable, achieving a survival rate of up to 67% of the seedlings. These findings reinforce the potential of this technique as a feasible alternative for the propagation and conservation of the species, with prospects for application in various biotechnological contexts.

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