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In vitro seed germination, seedling development and aclimattization of *Actinocephalus polyanthus* (bong.) Sano, an endemic everlasting flower species[1]

Sérgio Pedro Junior[2*], *Débora Perdigão Tejo*[2], *Gabriel Cruz Barata*[2], *Cristiano Medri*[3] *and Ricardo Tadeu de Faria*[2]

ABSTRACT

Actinocephalus polyanthus (bong.) Sano (Eriocaulaceae) is an everlasting endemic endemic to Brazil with great potential for use in landscaping and as a cut flower on the national and international markets. In order to ensure that its use as an ornamental plant does not jeopardise populations of the species in areas of natural occurrence, as well as to avoid predatory extraction, the development of protocols for its propagation is extremely important. However, no protocol for the in vitro propagation of *Actinocephalus polyanthus* has been reported in the literature. The aim of this study was to establish a protocol for the germination, seedling development and acclimatization of *A. polyanthus*. The use of Wood plant medium culture medium at concentrations of 75 and 100% proved to be the most effective for germinating the seeds, but it was not the best medium for growing the seedlings, given that for the growth phase the medium was MS at the highest concentrations, also 75 and 100% salt concentrations, for acclimatization the use of a shading screen proved to be the most effective and the use of washed sand or carolina soil were the best substrates.

Keywords: domestication, *in vitro* establishment, propagation, everlasting flower.

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[2] Universidade Estadual de Londrina, Centro de Ciências Agrárias, Departamento de Agronomia, Londrina, Paraná, Brazil. sergio.pedrojr@uel.br; deboratejo@hotmail.com; gabriel.cruz.barata@uel.br; faria@uel.br

[3] Universidade Estadual de Londrina, Centro de Ciências Biológicas, Departamento de biologia animal e vegetal, Londrina, Paraná, Brazil. cristianomedri@uel.br

*Corresponding author: sergio.pedrojr@uel.br

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INTRODUCTION

Brazil has the greatest plant biodiversity in the world, representing 17% of all terrestrial flora. Many of these species are ecologically important for biodiversity conservation, while others are of economic and socio-environmental importance, such as the evergreens of the Eriocaulaceae family. However, the propagation and cultivation of these species is considered a major challenge, as there are no established protocols for the propagation of most of them.[1]

Actinocephalus polyanthus (Bong.) Sano is an ornamental species endemic to Brazil, found in the states of Bahia, Goiás, Minas Gerais, Rio de Janeiro, São Paulo, Paraná, Rio Grande do Sul and Santa Catarina.[2-4] The flower stems of this plant can be preserved for several years without the need for preservative solutions, a characteristic that is highly valued in the cut flower market.[5] However, the trade in this species is almost entirely based on predatory wild harvesting, which can lead to genetic erosion and a reduction in natural populations.[6] In Paraná, the harvesting of wild plants for commercial purposes has been reported since 1976.[7]

In addition to its ornamental value, *A. polyanthus* plays an important ecological role, having interaction for at least 152 species of invertebrates, and is one of the most widespread Eriocaulaceae species.[8,9]

In Brazil, micropropagation has proven to be a viable biotechnological strategy for the production and conservation of rare and endangered species, such as the "everlastings". Notable species studied include *Comanthera mucugensis* subsp. Mucugensis,[10,11] *Syngonanthus elegantulus,*[12] *Syngonanthus elegans* (Bong.) Ruhland,[5] *Actinocephalus bongardii* (A. St.-Hil.) Sano,[13] *Comanthera curralensis* Moldenke[6] and *Paepalanthus chiquitensis* Herzog.[1] However, there are no reports in the literature on the in vitro propagation of *A. polyanthus.*

In vitro establishment is the first step in developing a micropropagation protocol for a species and can be carried out using various plant parts. In this study, seeds were chosen as the propagule type because they allow the maintenance of genetic variability and avoid the removal of individuals from their natural environment.[14]

Identifying the most suitable culture medium and the ideal salt concentrations for rapid and uniform seedling development in vitro is essential for production.[15] In addition, selection of the best substrate and lighting is crucial for seedling survival during transfer from the laboratory to the greenhouse, a process known as acclimatization.[16]

The aim of this study was therefore to develop a protocol for the in vitro propagation of *A. polyanthus*, to identify the most suitable culture medium and its optimum concentration for germination and initial growth, and to determine the most suitable lighting and substrate for acclimatization in the greenhouse. This is the first report in the literature on the propagation of this species.

MATERIAL AND METHODS

Collection of inflorescences, processing of diaspores and batch caracterization

Flower stalks were collected from five mother plants of *A. polyanthus* in April 2022 at Morro da Pedra Branca, Serra do Cadeado, Ortigueira-PR, Brazil (figure 1a). The physiological maturity of the fruit was characterized by the detachment of the petiole of the floral chapter from the umbel.

After collecting the material, the inflorescences were taken to the plant tissue culture laboratory at the Agricultural Sciences Center (CCA) of the State University of Londrina (UEL), Londrina-PR, Brazil, where they were dried for 7 days on kraft paper at room temperature. The chapters were stored in paper bags in a refrigerator at an average temperature of 10 ºC for 15 days. To process the seeds, the flowerheads were rubbed with a spatula on a petri dish (Figure 1b), then passed through a sieve with a mesh size of 0.4 mm and, finally, the floral remains were blown off with a De Leo© electric blower, with an opening of 2º (Figure 1c). After this process, to increase purity, the seeds were manually separated under a sterioscopic microscope (Figure 1d and e).

To characterize the batch, two replicates containing 100 seeds were dried in a forced circulation oven at 102 ºC for 17 hours, as recommended in the RAS for small seeds, and their water content was obtained. To obtain the viability of the batch, a tetrazolium test was carried out on 4 replicates of 50 seeds, in which they were soaked for 16 hours at 30 ºC. After this process, they were cut on the longitudinal axis in the median portion and placed to stain in 0.075% tetrazolium salt for 4 hours.[17] Viable seeds were those with a red colored embryo, as shown in figure 1f. The seed sample used had a water content of 63%; seed viability, measured by the tetrazolium test, was 92%; and the germination rate was 89%.

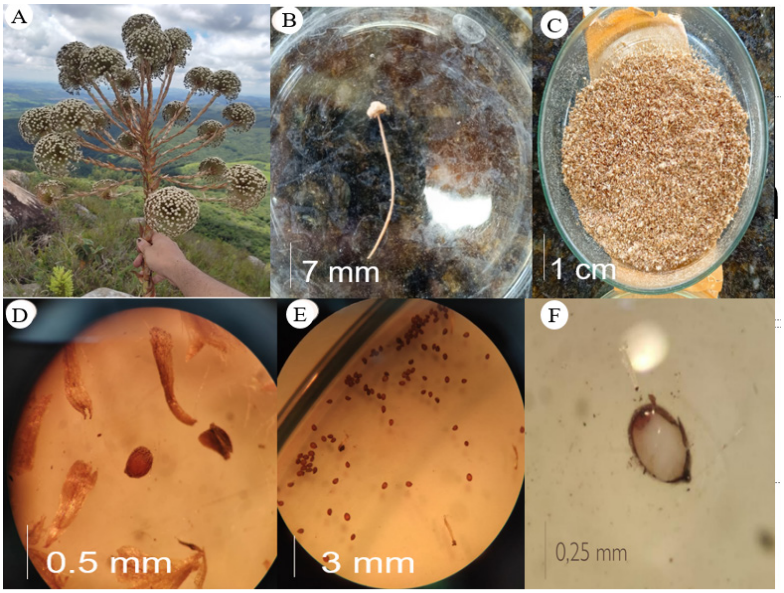

Figure 1. (a) Flower stem of *Actinocephalus polyanthus* (Bong.) Sano. (b). Beginning of seed processing of Actinocephalus polyanthus (Bong.) Sano, (c) flower heads after rubbing, sieving and blowing. (d) Flower heads after rubbing, sifting and blowing as seen under a stereoscopic microscope. (e) Fully processed seed after removal of impurities. (f) Viable seed of *Actinocephalus polyanthus* (Bong.) Sano. cut along the longitudinal axis showing a reddish embryo after the tetrazolium test, indicating a viable seed.

Culture medium and growing conditions

For both the seed germination experiment and the initial growth experiment, nine culture media were tested: (a) medium containing only deionized water, (b) Wood Plant Medium (WPM),[18] (c) modified WPM with 75% of the concentrations, (d) modified WPM with 50% of the concentrations, (e) modified WPM with 25% of the concentrations, (f) Murashige and Skoog (MS),[19] (g) MS modified with 75% of the concentrations, (h) MS modified with 50% of the concentrations, (i) MS modified with 25% of the concentrations. In all media, 6g L^{-1} of agar and 17g L^{-1} of sucrose were added.[5] The pH of the media was adjusted to 5.8 ± 0.2 before autoclaving, which was carried out for 20 minutes at 127 ºC and a pressure of 1.5atm. The flasks were kept in a growth room under irradiation of 47 µmol m^{-2} s^{-1} and a photoperiod of 16 hours of light and 8 hours of dark, at a constant temperature of 25 ± 2 ºC. The A. polyanthus seeds were separated and disinfected in a laminar flow chamber with 70% alcohol for 30 seconds, followed by immersion in 2% sodium hypochlorite for 10 minutes,[1] then triple washed in autoclaved deionized.

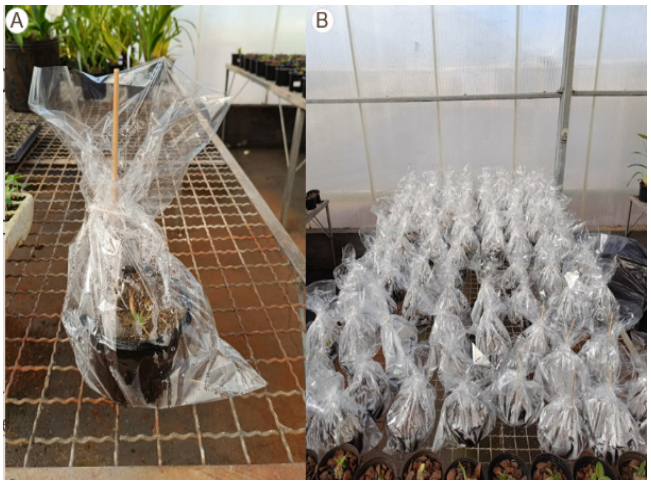

Figure 2. Acclimatization of Actinocephalus polyanthus (Bong.) Sano after 60 days of in vitro cultivation in pot 11. (b) Acclimatization system for A. polyanthus on the day the experiment was set up, using a polyethylene bag to maintain relative humidity; (b) Experiment set up in the greenhouse without shade.

Experimental design and data analysis

The nine media described above were used in the germination experiment, with four replicates, each replicate being a 250 ml glass jar containing 50 ml of each culture medium and 50 seeds per jar (figure 3a). The number of germinated seeds was analysed daily until stabilization for 7 consecutive days with no new germinated seeds, thus calculating the germinability (%GERM), the Germination Speed Index (GSI),[20] the mean germination time (MGT),[21] the first count (FC), carried out after 20 days and determined by the number of normal seedlings (plants with roots and aerial parts).[5] Seeds with a chlorophyll embryonic axis more than 2 mm long were considered germinated (figure 3b). The experimental design consisted of a 2 x 5 factorial design, with the first factor being the two formulations of culture medium used (WPM and MS) and factor 2 being the concentrations of the media (0, 25, 50, 75 and 100). The data obtained was subjected to analysis of homogeneity and variance (ANOVA) and the Tukey 5% probability test.

Seedling development

In the initial growth experiment, 40-day-old seedlings from culture medium (a) with a height of approximately 1 ± 0.2 cm were inoculated into the same nine culture mediums as mentioned above. Each treatment had 5 replicates, with each replicate being a 250 ml flask containing 50 ml of each culture medium with 10 plants in each flask. After 60 days of inoculating the explants, growth analyses were carried out on the seedlings in all the treatments (figure 3c): number of leaves (NF), aerial part length (APL), length of the largest leaf (LLL), number of live leaves (NLL), rosette diameter (RD), length of the largest root (LLR) and number of roots (NR). Size data was taken using a digital caliper with a precision of 0.001 cm. The experimental design consisted of a 2 x 5 double factorial, with the first factor being the two formulations of culture medium used (WPM and MS) and factor 2 being the concentrations of the media (0, 25, 50, 75 and 100). The data obtained was subjected to analysis of variance and the means were compared using Tukey's test at 5% probability. When the concentrations differed, regression analysis was carried out.

Acclimatization

A. polyanthus seedlings grown *in vitro* for 60 days on MS medium with 100% salt concentrations were transferred to plastic pots with 430ml of volume. To do this, the seedlings removed from the jars were washed in deionized water to completely remove the culture medium, taking care to keep the roots, and then placed in 4 different substrates: medium expanded vermiculite, Carolina Soil®, medium washed sand and a 1:1:1 mixture of the three above, all wrapped in transparent plastic bags to maintain internal humidity, as can be seen in figure 2a and b.[12]

These containers were then distributed in two vegetation houses with different light levels, one with a photon irradiance of 47 μmol m^{-2} s^{-1} and the other with an irradiance of 250 μmol m^{-2} s^{-1}. The plastic bags were cut laterally every 7 days until they were completely removed, which was done at 28 days.[13]

Watering was carried out twice a week by percolation during the first 28 days, when the pots were covered with a plastic bag to maintain humidity. After this period, with the

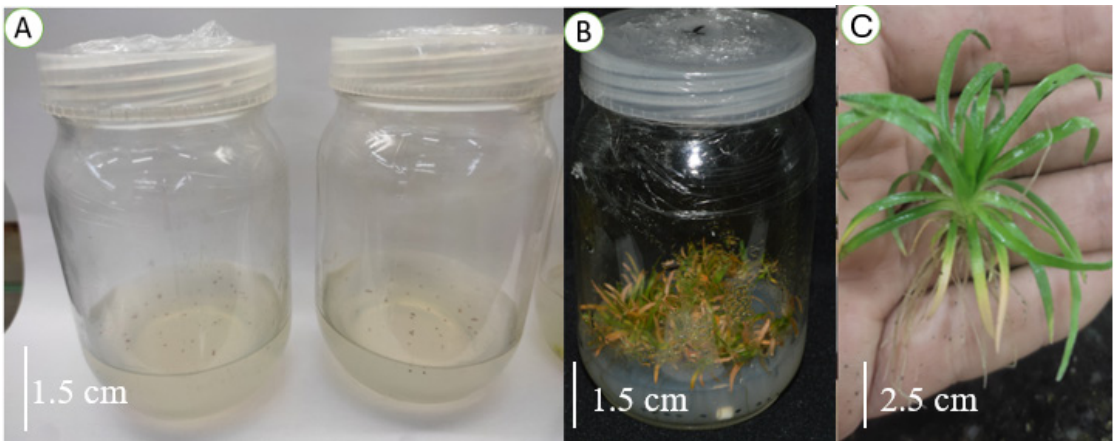

Figure 3. (a) 250ml flask containing 50ml of culture medium and 50 seeds per flask on the day the experiment was set up. (b) Flask containing 50ml of culture medium and 50 germinated seeds 30 days after the experiment was set up. (c) seedling after 60 days of initial growth in MS medium 100% of the salt concentration.

pots fully exposed to air, irrigation was performed manually every two days. This frequency was established based on the observation of a dry surface layer of the substrate, approximately 2 centimeters thick.

The acclimatization experiment consisted of 8 treatments, four different substrates and two light intensities, with 20 replicates per treatment, considering a pot with one plant a replicate. The data obtained was subjected to analysis of variance and the means were compared using Tukey's test at 5% probability.

RESULTS AND DISCUSSION

In vitro seed germination

The %GERM was influenced by the culture medium and the salt concentrations used. In the MS medium, the highest concentration (100%) had a lower germination than the others. On the other hand, the control (water agar) was superior to the others, as can be seen in Table 1.

The %GERM can be considered high compared to other Eriocaulaceae species. For example, *Actinocephalus bongardii* was also more responsive to the WPM medium, but had a germination rate of 74%, lower than that observed in this experiment. The germination rates of *A. polyanthus* analyzed in this work were also higher than those of *Syngonanthus elegantulus* (61%), *S. elegans* (63%) and *Paepalanthus chiquitensis* (82%), all from the family Eriocaulaceae.[1,5,12,13]

The observed results confirm those obtained by Pegô et al.[12] for *S. elegantulus* and by Albuquerque et al.[6] for *Comanthera curralensis*, where there was a decrease in germination as the concentration of salts in the MS medium increased, due to the higher concentration of osmotically active compounds, affecting the water available to the seeds.

GSI was influenced by the salt concentrations in the media. In MS, only the highest concentration (100%) differed negatively, being inferior to the others. In WPM, the highest GSI was obtained in the 75% treatment, which was higher than the others. This index can be used to determine the sensitivity of the seeds of a species to different concentrations of salts in the culture media and to identify the best conditions for the seeds to achieve the best expression of their physiological quality.[22]

MGT was also affected by salt concentrations in the culture media, following the same pattern as GSI. In MS, increasing concentrations had a negative effect; in WPM, the best performance was observed at 75%. In both media, the control treatment was statistically equal to the best concentration.

The increase in MGT and decrease in GVI in the MS medium may be due to the saturation of the salts present, as the seed imbibition process may be slower with increasing salt concentrations.[23] Winhelmann et al.,[24] who studied another evergreen species, *Angelonia integerrima*, found that the increased presence of mineral salts in the medium increased MGT, which affected the germination process, making it take longer and preventing the seeds from reaching their full physiological potential.

Table 1. Germination (%), germination speed index, mean germination time (days) and first count (%) *of Actinocephalus polyanthus* germinated *in vitro* after 38 days in two culture media (MS and WPM) at five different salt concentrations (0, 25, 50, 75 and 100%)

Culture médium	Salt concentration				
	0	25%	50%	75%	100%
	Germination (%)				
MS	81 a*	93 Aa	92 Aa	85 Ba	71 Bb
WPM	81 b	91 Aa	93 Aa	95 Aa	95 Aa
	Germination speed index				
MS	3,83 a	4,28 Aa	4,29 Aa	3,47 Ba	1,64 Bb
WPM	3,83b	3,98 Ab	4,19 Ab	4,62 Aa	3,92 Ab
	Mean germination time (days)				
MS	10,81 a	11,44 Aa	11,61 Ab	13,56 Bc	19,51 Bd
WPM	10,81 a	13,33 Bb	12,58 Bb	11,79 Aa	13,81 Ab
	First count (%)				
MS	76 a	88 Aa	88 Aa	76 Ba	38 Bb
WPM	76 b	78 Bb	88 Aa	94 Aa	86 Ab

* Equal uppercase letters between culture media and lowercase letters between salt concentrations do not differ statistically by the Tukey test ($P < 0.05$).

The pattern observed in other evergreens native to Brazil is not followed in most of the parameters analyzed in germination in this work, considering that the GVI in the WPM medium was not affected, as observed by Pegô et al.[5,12] in *S. elegantulus* and *S. elegans*, respectively, which may indicate a lower sensitivity of the species to the variation of nutrients in this medium.

The FC also shows that in the MS medium, increasing concentrations are detrimental to germination and subsequent formation of a normal seedling, as the highest concentration was the one with the lowest number of viable seedlings in this medium. In contrast, the WPM medium showed better performance, particularly at 75% and 50% concentrations. Notably, WPM medium at 75% or 100% salt concentration is recommended for in vitro germination of this species. It is also worth mentioning that MS at 25% has nearly the same ammonium and nitrate concentration as WPM at 100%, which may explain the comparable germination outcomes observed under these conditions.

In vitro seedling development

The MS medium with 100% salt concentration resulted in the greatest shoot growth (APL), with an average of 4.5 cm, which was higher than the 0, 25 and 50% salt concentrations and statistically equal to the 75% concentration (Table 4). The WPM medium had lower responses than the MS medium, but followed the same pattern where increasing concentrations led to greater growth of the aerial part. The concentration with the best results for this medium was 100%, with an average of 3 cm, which was higher than the others.

For the LLL in the MS medium, the concentrations of 50, 75 and 100 per cent were statistically superior to the others, with the highest average (3.95 cm) being observed in the 100 per cent concentration. For the WPM medium, the highest LLL was also obtained at 100% salt concentration, with an average of 2.45 cm, statistically different from the other concentrations (Table 2).

Table 2. Number of leaves (NL), Aerial part length (APL), largest leaf length (LLL), rosette diameter (RD), number of leaves (NL), number of roots (NR), number of live leaves (NLL) and largest root length (LRL) of *Actinocephalus polyanthus* (Bong.) Sano seedlings, after 60 days of initial in vitro growth in two culture media MS and WPM and in five salt concentrations

culture medium	salt concentration				
	0	**25%**	**50%**	**75%**	**100%**
			Number of leaves		
MS	3 b*	14 Aa	15 Aa	18 Aa	22 Aa
WPM	3 b	13 Aa	13 Aa	13 Ba	16 Ba
			Aerial part length (cm)		
MS	0,9 c	2.35 Ab	3.4 Ab	3.9 Aa	4.5 Aa
WPM	0,9 c	1,5 Ac	1.85 Bb	2 Bb	3 Ba
			Length of the largest leaf (cm)		
MS	0,9 c	2 Ab	3.1 Aa	3.55 Aa	3.95 Aa
WPM	0,9 c	1.3 Bc	1.5 Bb	1.75 Bb	2.45 Ba
			Number of live leaves		
MS	3 c	9 Ab	11 Ab	10 Ab	17 Aa
WPM	3 c	7 Ab	5 Bb	7 Bb	10 Ba
			Rosette diameter (mm)		
MS	12.53 d	27.33 Ac	38.83 Ab	51.53 Aa	55.55 Aa
WPM	12.53 d	19.16 Bc	25.75 Bb	26.58 Bb	31.26Ba
			Length of the largest root (cm)		
MS	0.6 b	2 Aa	2.45 Aa	2.5 Aa	1.9 Aa
WPM	0.6 b	1.9 Aa	1.5 Ba	2 Ba	1.5 Aa
			Number of roots		
MS	3 b	7 Aa	7 Aa	9 Aa	7 Aa
WPM	3 b	6 Aa	7 Aa	9 Aa	5 Ba

* Equal uppercase letters between culture media and lowercase letters between salt concentrations do not differ statistically by the Tukey test ($P < 0.05$).

Ivanova and Staden,[25] in their work with *Aloe polyphylla,* reported that increasing nitrogen concentrations is fundamental for growth parameters, a fact that was observed in this study, where the two mediums at the standard concentration showed the greatest growth when compared to the other concentrations diluted in the same culture medium. The higher levels of NH^{4+} found in the MS medium stimulated faster plant growth. Schnitzer *et al.* 2018, working with *Oncidium baueri*, also reported that increasing nitrogen concentrations is essential for better plant growth performance.[26]

The RD was also influenced by the salt concentrations. The MS medium once again proved to be more effective, with the best result obtained at a concentration of 100%, with an average of 55.55 mm, which was higher than the concentrations of 0, 25 and 50%. The WPM medium followed the same pattern as both media so far in all the growth characteristics, where with increasing salt concentrations there was greater development of the characteristic being analyzed. The 100 per cent concentration in the WPM medium showed the best results, with an average of 31.26 mm, higher than the 0, 25 and 50 per cent concentrations.

The parameters number of roots (NR), length of the largest root (LLL) and number of leaves (NL) showed differences when comparing the medium without added salts with the others, with the 0% concentration being lower than the others, 25, 50, 75 and 100%, which were statistically equal to each other.

The number of leaves was not affected by the concentrations of 25, 50, 75 and 100% in any of the media analyzed. However, the 0% concentration was lower than the others. However, for the parameter number of live leaves, the salt concentrations were responsive in both culture media, with the 100% concentration being the most effective and statistically superior to the others.

The fact that there was no change in the number of leaves with the increase in the concentration of salts in the medium may indicate that the plant grows easily in less favorable conditions, such as those found in high altitude fields. However, the increase in the number of live leaves (and consequent decrease in chlorotic leaves) is indicative of improved soil nutrition, meaning that nutrients such as nitrogen are translocated from the older leaves to the growth of the new leaves. The death of the oldest leaves of *A. polyanthus* has already been reported in the literature, but due to the lack of experiments with the cultivation of the species, it is not possible to say whether this is due to its own physiology or to the lack of nutrients in the field.[27]

The number of roots and the length of the largest root were not affected by any of the concentrations in either of the two media analyzed. In both media, the concentration with the best performance was 75 per cent, both for the number of roots and the length of the largest root.

The increase in salt concentrations resulted in greater growth of the seedlings *in vitro* and consequently the quality of the seedlings (Figures 4 and 5), with a growth pattern that was distinct in the *S. mucugensis, S. elegantulus* and *S. elegans* species, in which the WPM medium proved to be more effective.

In this study, the species showed greater growth on 100% MS medium, which shows that despite coming from an environment with sandy soils and low fertility, the plant is able to adapt well to laboratory conditions and assimilate the nutrients present in the culture medium

Acclimatization

30 days after the plants were acclimatized in a greenhouse with and without shade and different substrates, the survival rate was measured and is shown in Table 3.

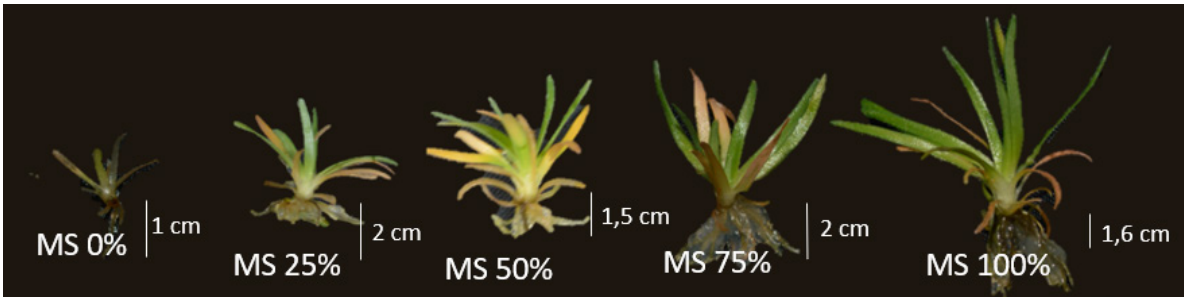

Figure 4. *Actinocephalus polyanthus* (Bong.) Sano seedlings after 60 days of in vitro growth in five culture media: MS 0%, MS 25%, MS 50%, MS 75% and MS 100% salt concentrations.

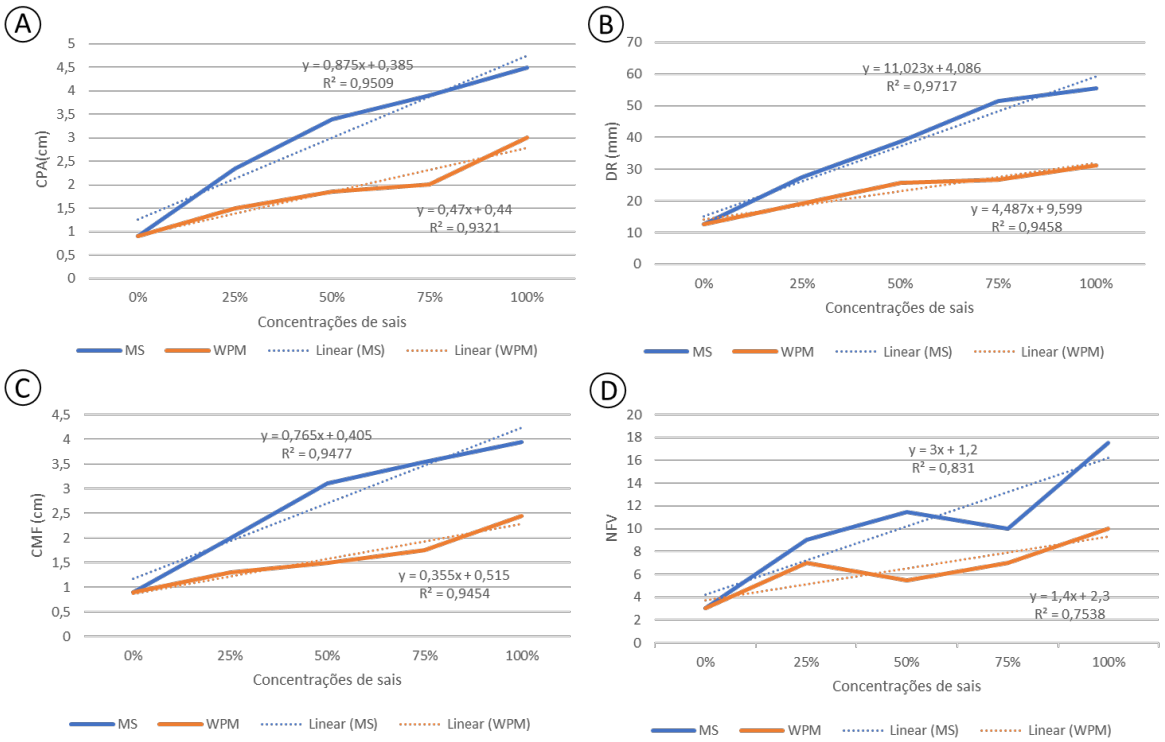

Figure 5. Different WPM (orange color) and MS (blue color) culture media at different salt concentrations (0, 25, 50, 75 and 100%) on growth parameters. A) Aerial part lenght (APL). B) Rosette diameter (RD). C) Length of the largest leaf (LLL) and D) number of live leaves (NLL).

Table 3. Percentage survival of *Actinnocephalus polyanthus* (Bong.) Sano seedlings after 30 days of acclimatization in a greenhouse in different substrates (washed sand, vermiculite, Carolina Soil and a 1:1:1 mixture of the three substrates mentioned above) and two types of lighting system (50% shade and full sun)

treatments	% survival
Full sun + washed sand	80% b
full sun + vermiculite	0% e
full sun + Carolina Soil	45% d
full sun + mixture	75% b
full sun + washed sand	100% a
full sun + vermiculite	50% c
full sun + Carolina Soil	95% a
full sun + mixture	100% a

In general, the half-shade treatments had the highest survival rates. The use of 50% shade combined with the use of washed sand or sand in a mixture as a substrate resulted in 100% survival. In both greenhouses, vermiculite was the worst substrate, which was also reported by Pegô et al. (2013) on *S. elegantulus*. The use of sand was effective in both types of greenhouses, with 80% survival in the one with plastic covering and 100% survival in the one with 50% shade. This may be due to the fact that the greenhouse is located in a highland field with sandy soil.

The greenhouse with 50% shade provided a higher survival rate, possibly due to the similarity in light intensity between the greenhouse and the growth room, bearing in mind that with increased luminosity it is possible that there is an increase in evapotranspiration, due to the lower thickness of the leaf cuticles, as well as non-functional stomata, less sun, less stress.

Another factor that may have interfered with the higher survival rate of the seedlings during acclimatization is the fact that in the field the young plants are not fully exposed to the sun, despite the country environment, because due to their small size they end up being shaded by larger shrubs and herbaceous plants. In practice, they initially grow in half shade.[27] This is the first report in the literature of a protocol involved in the production process of *A. polyanthus*, and the information in this article on the in vitro establishment of this species could contribute to the domestication and introduction of this beautiful native plant onto the market.

CONCLUSION

The use of WPM medium with 75% or 100% salt concentration is recommended for in vitro seed germination, while MS medium with 75 or 100% salt concentration is recommended for initial growth. For the acclimatisation

phase, a greenhouse with 50% shade and a substrate of washed sand or a mixture of sand with vermiculite and Carolina Soil© is recommended.

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DATA AVAILABILITY

The dataset generated and analyzed during the current study is available from the corresponding author, Sérgio Pedro Junior, on reasonable request. Public availability of the data is restricted due to the potential for significant physiological variability among seed batches.

AUTHOR CONTRIBUTIONS

Conceptualization: Débora Perdigão Tejo, Sérgio Pedro Junior.

Data curation: Gabriel Cruz Barata, Sérgio Pedro Junior.

Funding acquisition: Cristiano Medri, Ricardo Tadeu de Faria.

Methodology: Sérgio Pedro Junior.

Supervision: Cristiano Medri, Ricardo Tadeu de Faria.

REFERENCES

1. Gonçalves-Magalhães C, de Morais TP, de Santana DG, Asmar SA, Ribeiro-Oliveira JP, Luz JM. In vitro propagation of *Paepalanthus chiquitensis* Herzog (Eriocaulaceae), an endangered everlasting flower species. Plant Cell Tiss Organ Cult. 2023; 153:377–86. Available from: https://doi.org/10.1007/s11240-023-02475-w
2. Sano PT, Giulietti AM, Costa FN, Trovo M, Echternacht L, Tissot-Squalli ML, Watanabe MT, Hensold N, Andrino CO, Parra LR. Eriocaulaceae in Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. 2015.
3. Goebel G, Dechoum M, Castellani T. Guia sobre plantas nativas ornamentais de Restinga. Florianópolis: UFSC; 2019.
4. REFLORA – Herbário Virtual [Internet]. Rio de Janeiro: Jardim Botânico do Rio de Janeiro; c2025 [cited 2025 Jan 17]. Available from: https://floradobrasil.jbrj.gov.br/reflora/herbarioVirtual/
5. Pêgo RG, Paiva PD, Paiva R. Micropropagation protocol for *Syngonanthus elegans* (Bong.) Ruhland: an ornamental species. Acta Sci Agron. 2014; 36:347-53. Available from: https://doi.org/10.4025/actasciagron.v36i3.17946
6. Albuquerque MM, Brito AL, Lima AP, Alvim BF, Santana JR. In vitro establishment of *Comanthera curralensis*, "sempre viva" native of Chapada Diamantina-Bahia. Cienc. Rural. 2016; 46:991-5. Available from: https://doi.org/10.1590/0103-8478cr20131091
7. Castellani TT, D'eça-Neves FF. Population ecology of *Paepalanthus polyanthus*: predispersal hazards and seed production. Acta Bot. Bras.. 2000; 14:317-26. Available from: https://doi.org/10.1590/S0102-33062000000300008
8. Cereto C, Schmidt G, Martins A, Castellani T, Lopes B. Nesting of ants (Hymenoptera, Formicidae) in dead post-reproductive plants of *Actinocephalus polyanthus* (Eriocaulaceae), a herb of coastal dunes in southern Brazil. Insectes Soc. 2011; 58:469-71. Available from: https://doi.org/10.1007/s00040-011-0165-y
9. Martins AG. Uma planta, um universo: compreendendo a biodiversidade associada à sempre-viva-de-mil-flores, *Actinocephalus polyanthus* (Eriocaulaceae), em dunas no Sul do Brasil. [dissertation]. Santa Catarina: Centro de Ciências Biológicas, Universidade Federal de Santa Catarina; 2013.
10. Da Paixão Santos J, Dornelles AL, Pereira FD, Oliveira LM. Indução de calos em sempre-viva (*Syngonanthus mucugensis* Giulietti), utilizando diferentes tipos de explantes e concentrações de BAP. Acta Sci Biol Sci. 2008; 30(2):127-31.
11. Lima-Brito A, Resende SV, Lima CO, Alvim BM, Carneiro CE, Santana JR. In vitro morphogenesis of *Syngonanthus mucugensis* Giul: subsp. mucugensis. Cienc Agrotec. 2011; 35:502-10. Available from: https://doi.org/10.1590/S1413-70542011000300010
12. Pêgo RG, Paiva PD, Paiva R. Micropropagation of *Syngonanthus elegantulus*. Cienc Agrotec. 2013; 37:32-9. Available from: https://doi.org/10.1590/S1413-70542013000100004
13. Prudente DD, Nery FC, dos Reis MV, de Oliveira Paiva PD, Nery MC, Amin TD. In vitro germination and acclimatization of everlasting flower. Plant Cell Cult Micropropag. 2015; 11(2):62-9.
14. Unemoto LK, de Faria RT, Meneguce B, de Assis AM. Estabelecimento de um protocolo para a propagação in vitro de rainha-do-abismo, *Sinningia leucotricha* (Hoehne) Moore (Gesneriaceae). Acta Sci Agron. 2006;28(4):503-6.
15. Faria RT, Rodrigues FN, Oliveira LV, Müller C. In vitro *Dendrobium nobile* plant growth and rooting in different sucrose concentrations. Hortic Bras. 2004; 22:780-3. Available from: https://doi.org/10.1590/S0102-05362004000400023
16. Colombo RC, Aves GG, Bertoncelli DJ, Vero FS, Faria RT. In vitro multiplication and acclimatization of Heliconia hybrid (*Heliconia bihai* x *Heliconia caribaea* 'Jacquinii'). Agron Sci Biotechnol. 2016; 02(1):01-05. Available from: https://doi.org/10.33158/ASB.2016v2i1p1
17. Magalhães CG. Germinação de diásporos e micropropagação da sempre-viva *Paepalanthus chiquitensis* Herzog (Eriocaulaceae) [dissertation]. Bahia: Universidade Estadual de Feira de Santana; 2018.
18. Lloyd G, McCown B. Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot tip culture. Proc Int Plant Propag Soc. 1980; 30:421-7.
19. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant. 1962; 15(3):473-97.
20. Maguire JD. Speed of germination-aid in selection and evaluation for seedling emergence and vigour. Crop Sci. 1962; 2(1):176-7.
21. Labouriau LG. A germinação das sementes. Washington: Secretaria Geral da Organização dos Estados Americanos; 1983.
22. Ministério da Agricultura, Pecuária e Abastecimento. Regras para análise de sementes. Brasília: Mapa/ACS; 2009. 399p.
23. Pêgo RG, Paiva PD, Paiva R. In vitro establishment of *Syngonanthus paepalophyllus* in different culture media. Acta Hortic. 2015; 255-60. Available from: 10.17660/ActaHortic.2015.1083.31
24. Winhelmann MC, Tedesco M, Lucchese JR, Fior CS, Schafer G. Propagação in vitro de *Angelonia integerrima*. Rodriguésia. 2019; 70. Available from: https://doi.org/10.1590/2175-7860201970025
25. Ivanova M, Van Staden J. Nitrogen source, concentration, and NH_4^+: NO_3^- ratio influence shoot regeneration and hyperhydricity in tissue cultured *Aloe polyphylla*. Plant Cell Tissue and Organ Culture (PCTOC). 2009; 99(2):167-74.
26. Schnitzer JA, Brito OR, de Faria RT. Nitrogen fertilization in *Oncidium baueri* seedling growth. Afr J Agric Res. 2018;13(33):1747-53
27. Castellani TT, Scherer KZ, Paula GS. Population ecology of

Paepalanthus polyanthus (Bong.) Kunth: demography and life history of a sand dune monocarpic plant. Braz J Bot. 2001; 24:122-34. Available from: https://doi.org/10.1590/S0100-84042001000200001