Ciência Rural, Santa Maria, v.53:5, e20210599, 2023
http://doi.org/10.1590/0103-8478cr20210599
ISSNe 1678-4596
BIOLOGY

In vitro development and acclimatization of *Cyrtopodium aliciae* L. Linden & Rolfe, an endemic species of the Chapada Diamantina

Jardel de Oliveira[1] **Milena Cristina Moraes**[1] **Ceci Castilho Custódio**[1] **Nelson Barbosa Machado Neto**[1*]

[1]Faculdade de Ciências Agrárias, Universidade do Oeste Paulista (UNOESTE), 19067175, Presidente Prudente, SP, Brasil. E-mail: nbmneto@unoeste.br. *Corresponding author.

ABSTRACT: *Orchids are valued as ornamental plants, bioindicators, and medicinal plants, which implies that some species may be over-collected. Some inhabit very fragile environments and are under threat by the misuse of habitats and anthropogenic impacts. The search for beautiful plants and flowers has increased the number of facilities for micropropagation either by seeding or by cloning plants using in vitro techniques. However, not all species have appropriate media for growth and development that would help in conservation efforts.* Cyrtopodium aliciae *is an endemic species of rupestrian grassland in Brazil t. It has appeal as an ornamental plant or for use in hybridisation programs dueo its small size and white brownish-purple dotted flowers. This study compared three different media, namely ½ concentration Murashige and Skoog (MS), Vacin and Wendt, and Knudson C, during plant growth and their effect on the acclimatization of* Cyrtopodium aliciae*. The number and length of shoots and roots, increase in mass, and survival in vitro and ex vitro were analyzed. The experiment was conducted as completely random with a factorial arrangement of treatments (3 × 3) with 10 repetitions per treatment containing 10 plants for the in vitro experiment and 3 repetitions of 10 plants for the ex vitro experiment.* Cyrtopodium aliciae *performed better in the ½ concentration MS medium with a higher increase in mass, plant development, and survival under both in vitro and ex vitro conditions.*
Key words: *growth, culture media, conservation.*

Desenvolvimento *in vitro* e aclimatização de *Cyrtopodium aliciae* L. Linden & Rolfe: uma bela e endêmica espécie da Chapada Diamantina

RESUMO: *As orquídeas são valiosas como plantas ornamentais, bioindicadores ou medicinais, o que implica que algumas espécies podem ser coletadas em demasia. Algumas habitam ambientes muito frágeis e devido ao impacto antrópico, estão sob ameaça devido ao mau uso destes habitats. A busca por belas plantas e flores aumentou as facilidades para micropropagação, seja por semeadura ou por clonagem de plantas com técnicas in vitro. No entanto, nem todas as espécies possuem meios apropriados para crescimento e desenvolvimento que ajudem nos esforços de conservação.* Cyrtopodium aliciae *é uma bela espécie endêmica de campos rupestres do Brasil, apresenta bom apelo tanto como planta ornamental quanto como potencial para ser usada em programas de hibridização, devido ao seu pequeno tamanho de planta e belas flores brancas pontilhadas com marrom-púrpura. Este trabalho teve como objetivo comparar três meios diferentes, Murashige e Skoog à ½ concentração (MS); Vacin e Wendt (VW) e Knudson C (KC), durante o crescimento e na aclimatização. Foram analisadas as seguintes variáveis: número e comprimento de brotos e raízes, aumento de massa, sobrevivência in vitro e ex vitro. O experimento foi conduzido em delineamento inteiramente casualizado, com os tratamentos em um arranjo fatorial 3x3 (meios x tempo) com 10 repetições por tratamento contendo 10 plantas cada para a fase in vitro e três repetições com 10 plantas para a fase ex vitro.* Cyrtopodium aliciae *teve melhor desempenho na concentração de ½ MS com o maior aumento de massa, desenvolvimento da planta e sobrevivência tanto em condições in vitro quanto em condições ex vitro.*
Palavras-chave: *crescimento, meio de cultura, preservação.*

INTRODUCTION

The genus *Cyrtopodium* genus is widespread across the Americas, and its highest diversity occurs in Brazil (FLORA DO BRASIL, 2020). *Cyrtopodium* plants can be large, such as the *Cyrtopodium saintglerianum* (FLORA DO BRASIL, 2020) or *Cyrtopodium punctatum* (DUTRA et al., 2009), or small, such as *Cyrtopodium blanchetii* (FLORA DO BRASIL, 2020), which all have long and racemose inflorescences and are underutilised as ornamental plants. However, these plants have great potential as they can hybridise with other genera, such as *Galeandra*, *Cymbidium*, *Grammatophyllum*, or *Ansellia* in subtribe Cyrtopodinae or with the sister subtribe Catasetinae (*Catasetum*, *Cycnoches*,

Received 08.16.21 Approved 05.01.22 Returned by the author 07.11.22
CR-2021-0599.R1
Editors: Leandro Souza da Silva Ana da Silva Ledo

Mormodes, and *Clowesia*). Many are used as medicinal plants (ARAÚJO-LIMA et al., 2020) or as adhesives for many purposes. However, all these species face the risk of extinction, which is why their propagation is significant (DUTRA et al., 2009; SUZUKI et al., 2009). Cyrtopodium *aliciae* has an inflorescence crowded by white flowers with purplish-brown spots, bracts, sepals, floral segments with wavy margins, sympodial growth, and pseudobulbs. Moreover, fruit production occurs during the rainy season and ripening occurs during the dry season (SANTANA et al., 2016).

The world's flora consists of 250 000 (FLORA DO BRASIL, 2020) to 450 000 species (PIMM & JOPPA, 2015). Brazil has great plant biodiversity with more terrestrial species than many other countries (PIMM et al., 2010), harbouring approximately 35 700 species of Angiosperm and many threatened plant species (CNCFlora, 2021) and approximately 2700 species of orchids with approximately 1500 endemic species (FLORA DO BRASIL, 2020; ULLOA et al., 2017).

Orchids are valuable as ornamental or medicinal plants and are heavily collected for these reasons, thereby resulting in the transfer of wild germplasm to private collections (YAM & ARDITTI, 2009), which compromises the integrity of ecosystems. This environmental damage is difficult to repair and evaluate, especially with endangered species, because the scarcer the natural resource, the more difficult it is to return to its original state (RAMALHO & PIMENTA, 2010).

The commercialization of orchids has increased (HINSLEY et al., 2016, 2018) with the demand for other pot plants; the industry is an international business. Orchid cultivation is increasing from 6% to 9% annually in the global flower trade and can change the economic landscape of a country (SHARMA, 2019). In this context, Brazil needs to implement a quality model for the management of flowers and ornamental plant production (JUNQUEIRA & PEETZ, 2014). The growing importance of environmental preservation associated with the use of *in vitro* propagation techniques can minimize the effects of predatory collections of orchids, thereby allowing natural population maintenance and commercialization of propagated plants.

Orchid seeds consist of globular structures with few cells at maturity, very small undifferentiated embryos, and no reserve organs (LEE et al., 2008), and a lipid reserve. They do not have an appropriate enzyme system to convert lipid reserves into soluble sugars (LEE et al., 2006, 2008; MANNING & VAN, 1987; YAM & ARDITTI, 2009). They do not germinate properly; they develop into a structure called a protocorm, which later gives rise to roots and leaves (BASKIN & BASKIN, 2014). Under *in vitro* conditions, plant development occurs more quickly, thereby enabling the production of a greater quantity of more uniform seedlings in less time, in small places, and with a high phytosanitary attribute, minimising the extinction risk of these species (FARIA et al., 2006; MARTINI et al., 2001; SUZUKI & FERREIRA, 2007).

Although, much is known about germination and tissue culture, few attempts have been made with the genus *Cyrtopodium* (ARAÚJO, et al., 1999; DUTRA, et al., 2009; PEREIRA et al., 2015; PICOLOTTO et al., 2017; SOUSA et al., 2019) because it has been underutilised as an ornamental plant.

The change in the seedling development environment from *in vitro* to *ex vitro* triggers water stress, photosynthetic stress, changes in nutrient absorption, and phytosanitary problems (MORAES et al., 2009).

This study compared three different media during the *in vitro* and *ex vitro* micropropagation phases of *Cyrtopodium aliciae* for the production of this species given future multiplication, reintroduction in nature, and availability to the consumer market.

MATERIALS AND METHODS

The protocorms of *Cyrtopodium aliciae* were derived from Orquidário Aurora (Taciba, SP, Brazil) seeds germinated in ½ concentration Murashige and Skoog (MS) medium (SEATON et al., 2018).

The seeds were disinfected with a sodium dichloroisocyanurate (5 $g \cdot L^{-1}$) solution for 10 min, washed three times with autoclaved distilled water, and dispersed in ½ concentration MS medium (MURASHIGE; SKOOG, 1962) containing 20 $g \cdot L^{-1}$ of sucrose as the carbon source. The pH was corrected to 5.6 and autoclaved at 121 °C and 1 atm. The medium was dispensed on Falcon dishes (60 mm × 15 mm), and left in a chamber with controlled temperature until use. After sowing, the plates were sealed with polyvinyl chloride (PVC) film and kept in a growth chamber at a temperature of 23 ± 2 °C and a photoperiod of 16 h of light.

Growth experiments

Thirty-day-old seedlings from the germination procedures were used in three different media (Table 1), namely ½ concentration MS medium

Table 1 - Mineral composition of the media used in the development of the seedlings of *Cyrtopodium aliceae*.

----------Macronutrients (mM L^{-1})----------			
	Kc	½MS	VW
Ammonia (NH_4^+)	7.57	10.31	7.57
Nitrate (NO_3^-)	8.47	19.715	5.2
Phosphate (PO_4^{3-})	1.84	0.625	3.13
Potassium (K^+)	1.84	10.03	6.97
Sulphate (SO_4^{3-})	4.84	0.75	4.9
Calcium (Ca^{2+})	4.24	1.505	1.93
Magnesium (Mg^{2+})	1.02	0.75	1.02
Chlorine (Cl^-)	–	3.015	–
Nitrogen total	16.04	30.025	12.77
NH_4^+ :NO^{3-} ratio	0.89	0.523	1.456
----------Micronutrients (µM L^{-1})----------			
Manganese	42.319	47.679	42.319
Iron	24.89	24.89	22.63
Iodine	-	2.48	-
Molybdenum	0.11	0.34	-
Cupper	0.241	0.48	-
Boron	9.06	286.77	-
Zinc	1.013	13.16	-
Cobalt	-	1.61	-

Kc – Knuson C; ½ concentration MS - Murashige and Skoog; VW – Vacin and Went.

(MURASHIGE & SKOOG, 1962), Knudson C (KC) medium (KNUDSON, 1946), and Vacin and Wendt (VW) medium (VACIN & WENT, 1949), with 20 $g \cdot L^{-1}$ of sucrose as the carbon source and 8 $g \cdot L^{-1}$ of agar in each medium. The pH of the media was adjusted to 5.6. The media were distributed in 50 mL aliquots each into 269 mL baby food jars and autoclaved at 121 °C and 105 kPa for 15 min. Ten seedlings were placed in each jar for a total of 300 plants per treatment. The jars were maintained in a growth chamber with an irradiance of 35 µmol photons $\cdot m^{-2} \cdot s^{-1}$ at 25 ± 1 °C and 16 h of light.

The *in vitro* development was evaluated every 90 d via weighing the fresh mass and counting the shoots and roots. The seedlings were weighed individually, the structures were counted, and the seedlings were replanted in the same type of fresh medium. The flasks were closed with two layers of PVC film.

Acclimatization

At the end of the *in vitro* phase, the seedlings were removed from the jars, washed carefully to remove any agar debris, and immersed in a solution consisting of 2 $g \cdot L^{-1}$ of methyl thiophanate (a fungicide agent) for 1 h (MACHADO NETO, 2019). The solution was then removed, and the seedlings were allowed to dry overnight.

Acclimatization was performed in a netted house with 80% shading (Polysombra, Polysack) and a plastic cover. The conditions outside the greenhouse included an average maximum temperature of 28.2 °C, an average minimum temperature of 16.2 °C, and average relative humidity of 53.6%. The seedlings were planted in community pots with sphagnum moss and pine bark (<5 mm; 1:1 v:v) used as substrates. The seedlings were watered daily (15 $mm \cdot m^{-2}$) and fertigated weekly using 0.3 $g \cdot m^{-2}$ of hydrosoluble fertilizer (20:20:20 analysis) supplemented with 0.1 $g \cdot m^{-2}$ of $Ca(Cl_2)$ or $MgSO_4$ at two-week intervals. Seedling survival was evaluated after 90 and 180 d of *in vitro* cultivation according to the difference between the initial number and the final number of seedlings.

Survival and acclimatisation percentages

Survival and acclimatization percentages were calculated for *in vitro* and *ex vitro* conditions, respectively, as the percentage of the number of initial plants out of the final number of plants in each treatment.

Statistical analysis

The percentage of survival (%S) and acclimatization (%Ac) were transformed according to the equation $f(y) = arcsin\sqrt{x/100}$. The data were expressed

as mean values. The experiment was conducted in a completely random design and analysed as a factorial arrangement (3 × 3) with three different media (½ MS, KC, and VW media) and three periods of evaluation (90, 180, and 270 d) with 10 repetitions each, 10 seedlings per repetition for the *in vitro* experiment, and 3 repetitions of 10 plants each for the acclimatization experiment. The data were subjected to analysis of variance, and when significant, the means were compared using Tukey's test ($P \leq 0.05$) (FERREIRA, 2011).

RESULTS

No substantial growth was observed until 90 d of cultivation. There was a high growth of *Cyrtopodium aliciae* seedlings after 180 d of cultivation in the VW medium; however, after 270 d, the seedlings showing the greatest fresh weight were those growing in the ½ concentration MS medium (Figure 1). In the end, seedlings grown in both VW and KC media had an equivalent fresh weight but were twice as small as those grown in the ½ concentration MS medium.

The number of shoots obtained from different culture media are shown in figure 2A. After 180 d of cultivation, there was greater growth of shoots in ½ concentration MS medium, whereas there was no difference between that in the VW and KC media. At the end of the experiment, more shoots were grown in the ½ concentration MS medium, the number did not differ in the VW medium. The KC medium showed fewer shoots.

Figure 2B shows the roots growth data. After 180 d of cultivation, there was no difference in the number of roots in the VW and KC media; however, there was greater root development in the ½ concentration MS medium. After 270 d, plants that grew in the ½ concentration MS medium had twice the number of roots of seedlings that grew in the VM medium and had grown more than seedlings grown in the KC medium.

Figure 3 shows the root and leaf lengths of *Cyrtopodium aliciae* after 270 d of cultivation. The root length did not differ among treatments; however, there was a greater improvement in leaf length in the KC and ½ concentration MS media.

The ½ concentration MS medium was superior for fresh or dry weight in *Cyrtopodium aliciae* (Figure 4). The other media did not differ and produced lighter plants.

Differences were observed in the final *in vitro* survival. The ½ concentration MS medium had the highest survival rate, followed by the KC and VW media, which had different survival rates (Table 2). The *ex vitro* survival rate of *Cyrtopodium aliciae* differed in the three media analysed after 90 d (Table 2); plants derived from the ½ concentration MS medium had the highest survival rate, followed by the VW and KC media, which did not differ. After 180 d, plants derived from the ½ concentration MS medium had the highest survival rate, whereas those of plants derived

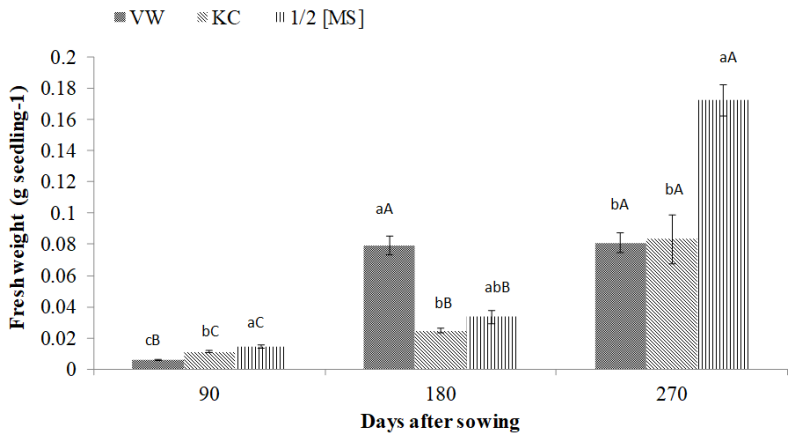

Figure 1 - Fresh weight increase per seedling (g) of *Cyrtopodium aliceae* at 90, 180 and 270 d of growth in ½ concentration Murashige and Skoog (MS), Vacin and Wendt (VW) and Knudson C (Kc) media. Similar uppercase letters for periods of evaluation in the same media and lowercase letters for media comparisons in the same period of evaluation are not different by Tukey's HSD test ($P < 0.05$).

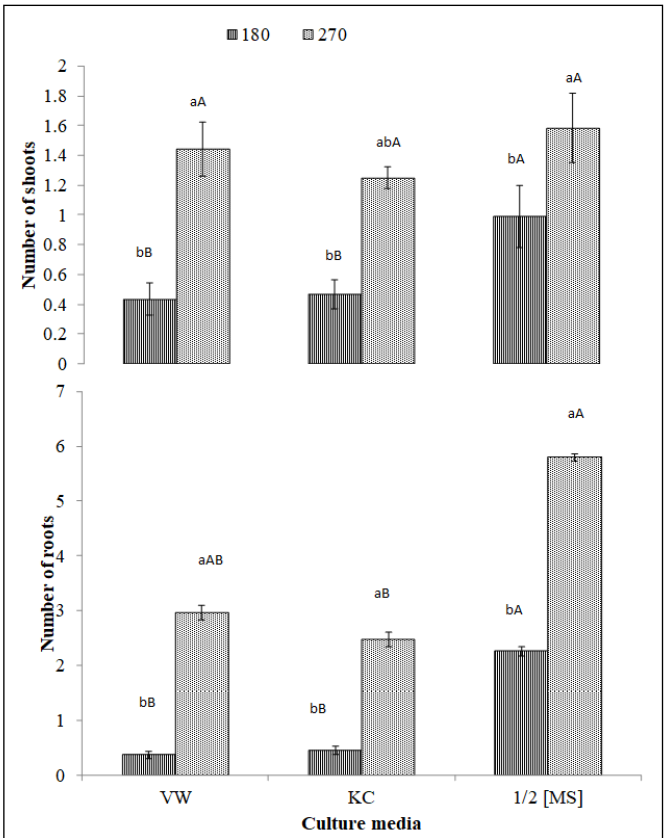

Figure 2 - Shoot (A) and root (B) number of *Cyrtopodium aliceae* seedlings at 180 and 270 d of culture in ½ concentration Murashige and Skoog (MS), Vacin and Wendt (VW) and Knudson C(Kc) media. Similar letters lowercase for time, and uppercase for media are not different by Tukey's HSD test ($P < 0.05$).

from the VW and KC media were significantly different. When the media were compared among times in the same medium, no differences were observed in the ½ concentration MS medium-derived plants. However, a decrease in the survival rate of greater than 20% in the KC medium and 28% in the VW medium was observed. The leaf length was superior for the ½ concentration MS medium-derived plants, followed by that of the VW and KC media-derived plants, which were different. The shoot length was superior for the ½ concentration MS medium-derived plants, while there were no differences between that of the other media-derived plants.

DISCUSSION

The largest development of *Cyrtopodium aliciae* seedlings were achieved in the medium rich in nitrogen and potassium, namely ½ concentration MS medium (Table 1), for all variables. *Cyrtopodium punctatum* plants were cultivated efficiently in a commercial medium (Sigma P723) containing a quarter of the MS salts supplemented with organics (DUTRA et al., 2009); however, it showed high germination in the ½ concentration MS medium, which was similar to that in the Malmgren (MALMGREN, 1992) and KC media.

Few studies have shown or compared the growth of *Cyrtopodium* species in synthetic media (DUTRA et al., 2009), compared growth regulators (RODRIGUES et al., 2015; VOGEL & MACEDO, 2011), or compared symbiotic germination (GUIMARÃES et al., 2013; PICOLOTTO et al., 2017; SOUSA et al., 2019). However, looking at the tribe Cymbidieae, some studies have been conducted on *Cymbidium* (GOGOI et al., 2011; MAHENDRAN

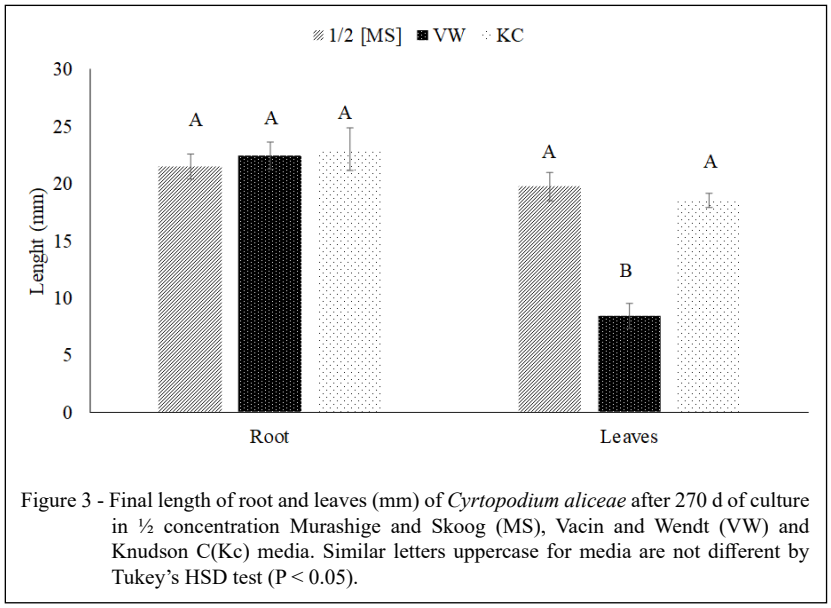

Figure 3 - Final length of root and leaves (mm) of *Cyrtopodium aliceae* after 270 d of culture in ½ concentration Murashige and Skoog (MS), Vacin and Wendt (VW) and Knudson C(Kc) media. Similar letters uppercase for media are not different by Tukey's HSD test ($P < 0.05$).

et al., 2013; MOHANTY et al., 2012) and have compared different media and *Catasetum* (FERREIRA et al., 2018).

The growth of shoots and roots and the accumulation of fresh mass were consistent throughout the cycle, except for the variables of leaf and root length. *Cattleya* seems to prefer MS medium over KC or other media (ABRÃO et al., 2014; JORGE et al., 2015; SUZUKI et al., 2009).

A study on *Cattleya warneri* grown for 180d on *in vitro* conditions showed that the MS medium provided significantly greater production of

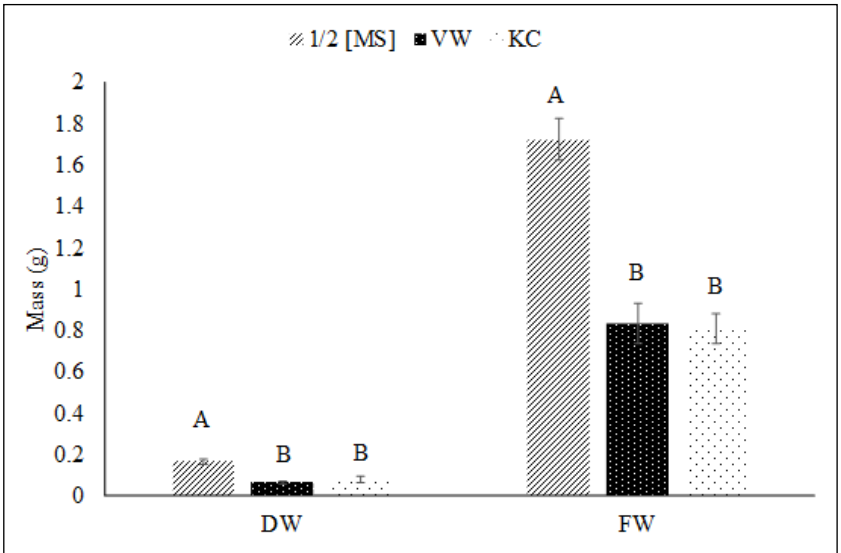

Figure 4 - Seedlings final dry and fresh weight (g) of *Cyrtopodium aliceae* after 270 d of culture in ½ concentration Murashige and Skoog (MS), Vacin and Wendt (VW) and Knudson C (Kc) media. Similar letters uppercase for media are not different by Tukey's HSD test ($P < 0.05$).

Table 2 - Survival and growth rate of shoots of *Cyrtopodium aliceae* seedlings from three media and cultures during acclimatization.

			----------Media----------		
			VW	½ [MS]	KC
Survival rate	*In vitro*		71.03±5.35b	91.43±1.90 a	67.86±5.48b
	Ex vitro	90d	86.67±3.33 bA	100.0±0.00 aA	86.67±8.82 bA
		180d	63.33±3.33 bB	96.67±3.33 aA	70.00±11.55 bB
Length (cm)		leaves	4.53±0.19 b	6.37±0.42 a	3.20±0.31 c
		shoot	3.93±0.39 b	8.02±0.23 a	4.26±0.45 b

Upper case letters within periods and lower case letters for different means for the same species indicate a significant difference ($P < 0.05$).

fresh mass than the KC and VW treatments (JORGE et al., 2015). The author also suggested that potassium acts in osmotic control in plants and has a greater concentration compared with that in other media, such as KC and VW media, thereby resulting in a lower fresh mass owing to reduced amounts of potassium. In contrast, KC medium proved to be more efficient in the initial development of protocorms after 120 d of sowing *in vitro*; however, after six months, the reduced concentration of potassium in the medium inhibited the accumulation of fresh mass in stems and roots. VW medium most effectively promoted the germination and growth of *C. bicolor* (SUZUKI et al., 2010). Shoot growth was higher in the MS medium but did not change in the VW medium. However, the KC medium showed lower shoot growth.

There was a reduction of more than 20% in the survival rate of *Cyrtopodium aliciae* at 180 d in the VW and KC media, whereas there was no change in that in the MS medium. The VW and KC media showed a reduction in shoot length (Table 2). Owing to the *in vitro* conditions, the remaining leaves reached senescence and/or did not survive the transplant, and each species had specificity and/or environmental conditions for acclimatization. According to FRANCO et al. (2007), leaf abscission is related to a plant strategy that reduces respiratory demands and reduces the exposed area of unnecessary structures to reduce the effects produced by different types of stress in terms of air humidity and the substrate itself.

CONCLUSION

The production of *Cyrtopodium aliciae* can be easily achieved by *in vitro* culture in the ½ concentration MS medium, which provides the toughest seedlings that perform better during acclimatisation.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest for this article. The founding sponsors had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, and in the decision to publish the results.

ACKNOWLEDGEMENTS

We would like to thanks CNPq for the DT Scholarship of NBMN and was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brasil - Finance code 001."

AUTHORS' CONTRIBUTIONS

Conceptualization: NBMN and JO. Data acquisition: JO. Design of methodology and data analysis: NBMN and CCC. All authors prepared the draft of the manuscript and critically revised the manuscript and approved of the final version.

REFERENCES

ABRÃO, M. C. R. et al. Germinação de sementes e desenvolvimento in vitro de plântulas de *Cattleya loddigesii* Lindl.(Orchidaceae). **Revista Brasileira de Biociências**, v.12, p.141–147, 2014. Available from: <http://www.ufrgs.br/seerbio/ojs/index.php/rbb/article/view/2838>. Accessed: Nov. 04, 2020

ARAÚJO, L. G. et al. Produção in vitro de mudas de *Cattleya walkeriana* e *Cyrtopodium palmifrons* a partir de sementes. **Pesquisa Agropecuária Tropical**, v.29, p.67–71, 1999. Available from: <https://www.revistas.ufg.br/pat/article/view/2852>. Accessed: Nov. 04, 2020. doi: 10.5216/pat.v29i2.2852.

ARAÚJO-LIMA, C. F. et al. Metabolomic analysis of *Cyrtopodium glutiniferum* extract by UHPLC-MS/MS and in vitro antiproliferative and genotoxicity assessment. **Journal of Ethnopharmacology**, v.253, p.112607, 2020. Available from: <https://www.sciencedirect.com/science/article/pii/S0378874119347312?casa_token=lntDsCL1-wQAAAAA:QOXM5u0Sni0zIptjN743tsOVJ2A7ujiTlsBp-c4Q40rk-tbhAoOXI3qFJJ0K5lFQ8n7cLOijq-A>. Accessed: Mar. 03, 2021. doi: 10.1016/j.jep.2020.112607.

BASKIN, C. C.; BASKIN, J. M. **Seeds**: ecology, biogeography and evolution of dormancy and germination. Academic Press: San Diego, CA, USA. 2014.

BRASIL. **Flora do Brasil 2020 em construção**. Jardim Botânico do Rio de Janeiro. [s.l: s.n.], 2020. Available from: <http://floradobrasil.jbrj.gov.br/>. Accessed: Nov. 04, 2020.

GOGOI, K. et al. A. Asymbiotic seed germination and in vitro seedling development of *Cymbidium aloifolium* (L.) Sw.: a multipurpose orchid. **Journal of Plant Biochemistry and Biotechnology**, v.20, p.90–95, 2011. Available from: <https://link.springer.com/article/10.1007/s13562-010-0031-4>. Accessed: Mar. 04, 2021. doi: 10.1007/s13562-010-0031-4.

DUTRA, D. et al. Asymbiotic seed germination and in vitro seedling development of *Cyrtopodium punctatum*: a propagation protocol for an endangered Florida native orchid. **Plant Cell, Tissue and Organ Culture**, v.96, p.235–243, 2009. Available from: <https://www.sciencedirect.com/science/article/pii/S0011224007000557?casa_AA:JlKbQgP2j5cyakwUvFzypiQKjw2eW8svG6OVihByOpp>. Accessed: Mar. 4, 2020. doi: 10.1007/s11240-008-9480-z.

FARIA, R. T. et al. Propagação in vitro de *Oncidium baueri* Lindl. (*Orchidaceae*) sem uso de ágar. **Acta Scientiarum. Agronomy**, v.28, p.71–74, 2006. Available from: <https://periodicos.uem.br/ojs/index.php/ActaSciAgron/article/view/1672>. Accessed: Nov. 04, 2020. doi: 10.4025/actasciagron.v28i1.1672.

GUIMARÃES, F. A. R. et al. Symbiotic propagation of seedlings of *Cyrtopodium glutiniferum* Raddi (*Orchidaceae*). **Acta Botanica Brasilica**, v.27, p.590–596, 2013. Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0102-33062013000300016&lng=en&tlng=en>. Accessed: Nov. 04, 2020. doi: 10.1590/S0102-33062013000300016.

JORGE, J. et al.. Germinação e crescimento inicial in vitro de *Cattleya warneri* T. Moore (Orchidaceae). **Revista Brasileira de Biociências**, v.13, p.131–141, 2015. Available from: <http://www.ufrgs.br/seerbio/ojs/index.php/rbb/article/view/3083>. Accessed: Nov. 04, 2020.

JUNQUEIRA, A. H.; PEETZ, M. D. S. O setor produtivo de flores e plantas ornamentais do Brasil, no período de 2008 a 2013: atualizações, balanços e perspectivas. **Revista Brasileira de Horticultura Ornamental**, v.20, p.115, nov. 2014. Available from: <http://rbho.emnuvens.com.br/rbho/article/view/727>. Accessed: Nov. 04, 2020. doi: 10.14295/rbho.v20i2.727.

LEE, Y.-I. et al. Embryo development in the lady's slipper orchid, *Paphiopedilum delenatii*, with emphasis on the ultrastructure of the suspensor. **Annals of Botany**, v.98, p.1311–1319, 2006. Available from: <https://academic.oup.com/aob/article-abstract/98/6/1311/195174>. Accessed: Nov. 04, 2020. doi: 10.1093/aob/mcl222.

LEE, Y.-I. et al. Embryology of *Phalaenopsis amabilis* var. formosa: embryo development. **Botanical Studies**, v.49, p.139–146, 2008. Available from: <http://static2.wikia.nocookie.net/__cb20091018105041/orchids/en/images/6/6c/Embryology_of_Phalaenopsis_amabilis_var._formosa-.pdf>. Accessed: Nov. 04, 2020.

MACHADO NETO, N. B. Selection Parameters of a new "coerulea" multiflora hybrid: Cattlianthe Aurora's Blue Pride. **Crop Breeding and Applied Biotechnology**, v.19, p.487–490. 2019. Available from: <https://www.scielo.br/j/cbab/a/kPJPCFV6rSZMbYLzdgmcSKJ/?lang=en> Accessed: Nov 04, 2020. doi: 10.1590/1984-70332019v19n4c70.

MAHENDRAN, G. et al. Asymbiotic seed germination of *Cymbidium bicolor* Lindl. (Orchidaceae) and the influence of mycorrhizal fungus on seedling development. **Acta Physiologiae Plantarum**, v.35, p.829–840, 2013. Available from: <https://idp.springer.com/authorize/casa?redirect_uri=https://link.springer.com/content/pdf/10.1007/s11738-012-1127-3.pdf&c>. Accessed: Nov. 04, 2020. doi: 10.1007/s11738-012-1127-3.

MALMGREN, S. Crossing and cultivation experiments with Swedish orchids. **Svensk Botanisk Tidskrift**, v.86, p.337–346, 1992.

MANNING, J. C.; VAN STADEN. J. The development and mobilisation of seed reserves in some African orchids. **Australian Journal of Botany**, v.35, p.343–353, 1987. Available from: <http://www.publish.csiro.au/BT/BT9870343>. Accessed: Nov. 04, 2020. doi: 10.1071/BT9870343.

MARTINI, P. C. et al. Propagação de orquídea *Gongora quinquenervis* por semeadura in vitro. **Pesquisa Agropecuária Brasileira**, v.36, p.1319–1324, 2001. Available from: <https://www.scielo.br/j/pab/a/PmCm9njXK5V68JFpfyRPnqt/?lang=pt>. Accessed: Nov. 04, 2020. doi: 10.1590/S0100-204X2001001000015.

MOHANTY, P. et al. A simple and efficient protocol for the mass propagation of *Cymbidium mastersii*: an ornamental orchid of Northeast India. **AoB Plants**, v.2012, 2012. Available from: <https://academic.oup.com/aobpla/article/doi/10.1093/aobpla/pls023/176762>. Accessed: Feb. 16, 2021. doi: 10.1093/aobpla/pls023.

MORAES, C. P. et al. Desenvolvimento in vitro de *Cattleya tigrina* A. Richard (Orchidaceae) utilizando fertilizantes comerciais. **Ensaios e Ciência: Ciências biológicas, agrárias e da saúde**, v.13, p.57-65., 2009. Available from: <https://www.redalyc.org/pdf/260/26015684007.pdf>. Accessed: Nov. 04, 2020.

MURASHIGE, T.; SKOOG, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. **Physiologia Plantarum**, v.15, p.473–497, 1962. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1399-3054.1962.tb08052.x>. Accessed: Nov. 04, 2020. doi:10.1111/j.1399-3054.1962.tb08052.x.

PEREIRA, M. C. et al. Characterization of seed germination and protocorm development of *Cyrtopodium glutiniferum* (Orchidaceae) promoted by mycorrhizal fungi *Epulorhiza* spp. **Acta Botanica Brasilica**, v.29, p.567–574, dez. 2015. Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0102-33062015000400567&lng=en&tlng=en>. Accessed: Nov. 04, 2020. doi: 10.1590/0102-33062015abb0078.

PICOLOTTO, D. R. N. et al. Micropropagation of *Cyrtopodium paludicolum* (Orchidaceae) from root tip explants. **Crop Breeding and Applied Biotechnology**, v.17, p.191-197, 2017. Available from: <https://www.scielo.br/j/cbab/a/vw4wYvBBYWPVDVBM5XKwpGn/?lang=en>. Accessed: Jun. 16, 2021. doi: 10.1590/1984-70332017v17n3a30.

PIMM, S. L. et al. How many endangered species remain to be discovered in Brazil. **Natureza & Conservação**, v.8, p.71–77, 2010. Available from: <http://doi.editoracubo.com.br/10.4322/natcon.00801011>. Accessed: Nov. 04, 2020. doi:10.4322/natcon.00801011.

PIMM, S. L.; JOPPA, L. N. How many plant species are there, where are they, and at what rate are they going extinct? **Annals of**

the Missouri Botanical Garden, v.100, p.170-176, 2015. Available from: <https://bioone.org/journals/annals-of-the-missouri-botanical-garden/volume-100/issue-3/2012018/How-Many-Plant-Species-are-There-Where-are-They-and/10.3417/2012018.full>. Accessed: Nov. 17, 2020. doi: 10.3417/2012018.

RAMALHO, A.; PIMENTA, H. Valoração econômica aplicada à extração ilegal da orquídea *Cattleya granulosa* no parque natural Dom Nivaldo Monte de natal/RN. **Holos**, v.1, p.62-82, 2010. Available from: <https://www2.ifrn.edu.br/ojs/index.php/HOLOS/article/view/333>. Accessed: Nov. 17, 2020. doi: 10.15628/holos.2010.333.

RODRIGUES, L. A. et al. *In vitro* propagation of *Cyrtopodium saintlegerianum* Rchb. F. (*Orchidaceae*), a native orchid of the Brazilian savannah. **Crop Breeding & Applied Biotechnology**, v.15, p.10–17, 2015. Available from: <https://www.scielo.br/j/cbab/a/xJ3jr6XksV5V9BhbsPBsdzt/?lang=en>. Accessed: Nov 03, 2020. doi: 10.1590/1984-70332015v15n1a2.

SANTANA, K. C. et al. Phenodynamics of five orchids species growing on rock outcrops in the Chapada Diamantina Mountains in northeastern Brazil. **Acta Botanica Brasilica**, v.30, n.3, p.508–513, 2016. Available from: <https://www.scielo.br/j/abb/a/DQt9TVJXsgHRKNn8HvwzvNt/?lang=en>. Accessed: Nov. 04, 2020. doi: 10.1590/0102-33062016abb0132.

SOUSA, K. C. I. et al. Seed germination and development of orchid seedlings (*Cyrtopodium saintlegerianum*) with fungi. **Rodriguésia**, v.70, p.e02302016, 2019. Available from: <https://www.scielo.br/j/rod/a/BDTMDgnzwcK9MVnG5pWgM6q/?lang=en>. Accessed: Nov. 03, 2020. doi: 10.1590/2175-7860201970004.

SUZUKI, R. M. et al. Germinação e crescimento in vitro de *Cattleya bicolor* Lindley (Orchidaceae). **Hoehnea**, v.34, p.731–742, 2010. Available from: <https://www.scielo.br/j/hoehnea/a/L3rMgHYSRXcqXTttwfNhWnP/?format=pdf&lang=pt>. Accessed: Nov. 04, 2020. doi: 10.1590/S2236-89062010000400004.

SUZUKI, R. M. et al. In vitro germination and growth of *Hadrolaelia tenebrosa* (Rolfe) Chiron & VP Castro (Orchidaceae), an endangered species of the Brazilian flora. **Hoehnea**, v.36, p.657–666, 2009. Available from: <https://www.scielo.br/j/hoehnea/a/kTq3jc84fZVymrkhvhYLctG/?lang=pt>. Accessed: Nov. 04, 2020. doi: 10.1590/S2236-89062009000400006.

SUZUKI, R. M.; FERREIRA, W. M. Introdução às técnicas de micropropagação de orquídeas. A Botânica no Brasil: pesquisa, ensino e políticas públicas ambientais. **Imprensa Oficial, São Paulo**, p.655–659, 2007.

ULLOA, C. U. et al. An integrated assessment of the vascular plant species of the Americas. **Science**, v.358, p.1614–1617, 2017. Available from: <https://science.sciencemag.org/content/358/6370/1614>. Accessed: Nov. 04, 2020. doi: 10.1126/science.aao0398.

VOGEL, I. N.; MACEDO, A. F. Influence of IAA, TDZ, and light quality on asymbiotic germination, protocorm formation, and plantlet development of *Cyrtopodium glutiniferum* Raddi., a medicinal orchid. **Plant Cell, Tissue and Organ Culture**, v.104, p.147–155, 2011. Available from: <https://link.springer.com/article/10.1007/s11240-010-9810-9>. Accessed: Nov. 03, 2020. doi: 10.1007/s11240-010-9810-9.

YAM, T. W.; ARDITTI, J. History of orchid propagation: a mirror of the history of biotechnology. **Plant Biotechnology Reports**, v.3, p.1–56, 2009. Available from: <http://link.springer.com/10.1007/s11816-008-0066-3>. Accessed: Nov. 04, 2020. doi: 10.1007/s11816-008-0066-3.