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ORIGINAL ARTICLE

In vitro germination of seeds and acclimatization of Macambira (*Bromelia laciniosa* Martius ex Schultes f.) seedlings

Germinação in vitro de sementes e aclimatização de mudas de Macambira (Bromelia laciniosa Martius ex Schultes f.)

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ABSTRACT: The macambira is an ornamental plant that has a high degree of ecological importance. The objective in this research was to evaluate the in vitro germination of macambira in different culture media and establish the best substrate for the acclimatization of micropropagated plants. Five treatments were tested: 0, 25, 50, 75, and 100% salt of Murashige and Skoog (MS) medium. We calculated the percentage of germination and germination speed index (GSI). For testing acclimatization, five combinations of substrates were evaluated: coconut coir : earthworm castings (2:1); coconut coir : earthworm castings: sand (1:1:1, 2:1:1 and 2:2:1); and vermiculite : earthworm castings (2:1). There was no difference between the concentrations of MS salts based on the percentage of germination. For the GSI, the treatment 100% MS salts had the poorest results. There was no significant difference in the acclimatization of seedlings for the variables number of leaves and dried mass of roots and shoots. For the variable length of the longest root, coconut coir: earthworm castings (2:1) and coconut coir : earthworm castings : sand (2:1:1 and 2:2:1) had the highest averages, and for dried root mass, the coconut coir : earthworm castings : sand (1:1:1 and 2:2:1) provided the best results. Germination can be carried out *in vitro* in a medium containing only water and agar, and for acclimatization, the substrate coconut coir : earthworm castings : sand (2:2:1) should be used.

RESUMO: *A macambira é uma planta ornamental, que apresenta elevado grau de importância ecológica nos ecossistemas. O objetivo deste trabalho foi avaliar a germinação in vitro da macambira em função de diferentes meios de cultura, além de estabelecer o melhor substrato para a aclimatização de mudas micropropagadas. Para tanto, foram testados cinco tratamentos (DIC), com 100, 75, 50, 25 e 0% dos sais do meio MS. Foi estimada a porcentagem de germinação e verificado o índice de velocidade de germinação (IVG). Para aclimatização, foram avaliadas cinco combinações de substratos: pó de coco : húmus de minhoca (2:1); pó de coco : húmus de minhoca : areia (1:1:1), (2:1:1), (2:2:1), e vermiculita: húmus de minhoca (2:1). Não houve diferença entre as concentrações de sais MS quanto à porcentagem de germinação. Quanto ao IVG, o tratamento 100% de sais MS apresentou o pior resultado. Na aclimatização das mudas, para as variáveis 'número de folhas e de raízes' e 'matéria seca de parte aérea', não houve diferença. Para a variável 'comprimento da maior raiz', os substratos pó de coco : húmus de minhoca (2:1) e pó de coco : húmus de minhoca : areia (2:1:1) e (2:2:1) apresentaram as maiores médias e, para matéria seca de raízes, os substratos pó de coco : húmus de minhoca : areia (1:1:1) e (2:2:1) proporcionaram os melhores resultados. A germinação in vitro pode ser realizada em meio contendo apenas água e ágar; para a aclimatização, deve-se utilizar o substrato pó de coco : húmus de minhoca : areia (2:2:1).*

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1 Introduction

The macambira (*Bromelia laciniosa* Martius ex Schultes f.) is an ornamental plant belonging to the Bromeliaceae family, with 60 genera and 3170 species. Individuals of this family can be found across the Americas, and approximately 40% of known species are present in Brazil. Most of these species are located in the dry areas of the northeast of Brazil, from Bahia to Piauí; they are of high ecological importance in the ecosystems in which they are present and affect many aspects of the ecosystem they inhabit (WANDERLEY et al., 2009).

In vitro culture of bromeliads has been used for commercial purposes and has gained importance in the preservation of rare or endangered species (RECH FILHO et al., 2005). The main advantages of *in vitro* cultivation of seeds are the possibility of generating a large number of mother plants, avoiding the removal of individuals from nature (aiding in the conservation of endangered species), ensuring genetic variability, and supplying the landscape and floral markets (PEREIRA et al., 2011).

Techniques to induce higher germination and physiologic quality are important for increasing the performance and potential of the seeds *in vitro* and, therefore, the uniformity of plants under field conditions (ARAGÃO et al., 2003).

The nutritional requirements in micropropagation vary according to species. Studies to identify the culture media that promote *in vitro* germination are of great importance because obtaining a large number of seedlings with high genetic and phytosanitary quality is desirable (PINHEIRO et al., 2001). There are studies on developing efficient protocols for *in vitro* germination of diverse cultures, including bromeliads (LÉDO et al., 2007). However, the biggest problem restricting the use of *in vitro* culture on a commercial scale is the low survival rate of seedlings produced by this technique during the acclimatization process. The main cause of seedling mortality during micropropagation is the stress caused by the excessive water loss of seedlings (DUTRA; WENDLING; BRONDANI, 2009). Thus, the selection of the substrate is of fundamental importance in the growth and development of micropropagated plants and directly influences the success of this step (WAGNER JÚNIOR et al., 2003). Therefore choosing the best substrate for a propagated species depends on the plant's needs or how it can be manipulated.

The aim of this study was to evaluate *in vitro* germination of macambira seeds using different culture media, as well as to establish the best substrate for acclimatization of the seedlings.

2 Materials and Methods

The assays were carried out in the Plant Tissue Culture and Breeding Laboratory of the Agronomic Engineering Department of the Federal University of Sergipe in the city of São Cristóvão, Sergipe State, Brazil.

For the germination test, 30 g L^{-1} of sucrose and 7 g L^{-1} of agar were added to the culture medium. The medium pH was adjusted to 5.8±0.1, and the media were placed in an autoclave for 15 min (at 121±1 °C and a pressure of 1.05 atm). Using a scalpel, constantly flamed, the seeds were inoculated into vials. The seeds were washed in a Tween 20® solution (2 drops/100 mL) and rinsed in running water for 30 min. In a laminar flow chamber, seeds were submerged in 92.8º ethyl alcohol for 2 min followed by 2.5% sodium hypochlorite for 5 min. After triple washing with distilled and autoclaved water, seeds were isolated in the culture medium.

The cultures were kept in growth chambers at a controlled temperature of 25±2 °C, with a photoperiod of 12 hours and a light intensity of 60 μmol m^{-2} s^{-1}, using cool white fluorescent light.

Five treatments were tested in a completely randomized design; 0 (water and agar), 25, 50, 75, and 100% salt of Murashige and Skoog (MS) medium, with 10 replications and 10 seeds per replication. Seed germination was evaluated daily until the data were repeated for four consecutive days; the germination percentage and germination speed index (GSI) were calculated over 24 days.

In vitro produced macambira seedlings were used; they were washed in running water to eliminate any culture medium adhering to the roots and then transferred to plastic trays. The plastic trays had 162 cells of 70 cm^3, which contained the different treatments and were maintained in a greenhouse covered with a 50% shade screen, and an intermittent irrigation system was used to ensure a high relative humidity.

The experiment was conducted in a completely randomized block design with five replications and five plants per replication. Five combinations of substrates were tested: coconut coir : earthworm castings (2:1); coconut coir: earthworm castings : sand (1:1:1, 2:1:1 and 2:2:1); and vermiculite : earthworm castings (2:1). To all the substrates, we added 1 g L^{-1} of limestone.

After 60 days of acclimatization, we evaluated the number of leaves and roots, the length of the longest root, and the dry weight of aerial parts and roots.

Data were subjected to analysis of variance, and means were compared by Tukey's test at 5% probability using the software Sisvar ® (FERREIRA, 2011).

3 Results and Discussion

There was no difference between the concentrations of MS salts for germination percentage; the average germination was 93%. In conducting research with *Pitcairnia flammea*, Pereira et al. (2011) found similar results in testing variations of MS salts and activated carbon.

For the GSI, the treatments 0, 25, and 50% salt of MS medium salts showed the best results, with indexes ranging from 0.60 to 0.62 (Figure 1). Reis et al. (2008), studying the *in vitro* germination of *Melissa officinalis* L. seeds, found the lowest values for the same variable when 100% MS salts was used. According to these authors, it is possible that the high concentration of salts in the medium affected the osmotic potential and, consequently, the water availability for imbibition of seeds during germination. No differences in the germination percentages of *Neoregelia mucugensis* Leme and *Orthophytum mucugense* Wand were observed when 50 to 100% MS salts were used (BELLINTANI et al., 2007).

For the acclimatization of seedlings, the variables number of leaves, number of roots and dried mass of aerial parts averaged 5.44, 4.52 and 127.64 mg, respectively; there was no difference between the substrates studied (Table 1). For

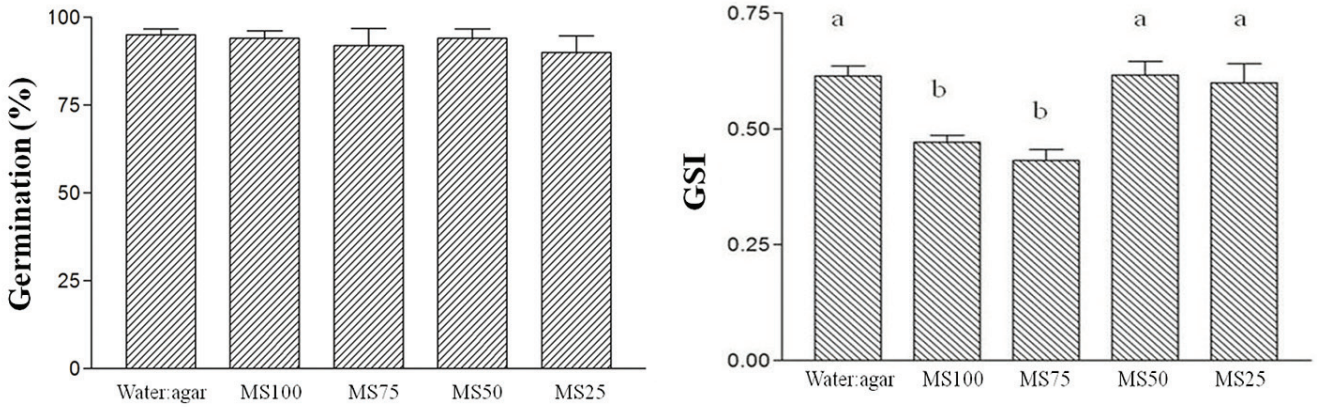

Figure 1. *In vitro* germination and germination speed index (GSI) of macambira seeds. MS100= MS medium with 100% salts; MS75= MS medium with 75% salts; MS50= MS medium with 50% salts and MS25= MS medium with 25% salts. Same letters do not differ by Tukey's test ($p < 0.05$).

Table 1. Number of leaves, number of roots, length of the longest root, and dry weight of aerial parts and roots after 60 days of acclimatization.

Substrates	Number of leaves
CC+EC (2:1)	5.54 a
CC+EC+S (1:1:1)	5.44 a
CC+EC+S (2:1:1)	5.36 a
CC+EC+S (2:2:1)	5.44 a
V+EC (2:1)	5.44 a
CV (%)	6.59
	Number of roots
CC+EC (2:1)	4.29 a
CC+EC+S (1:1:1)	4.72 a
CC+EC+S (2:1:1)	4.72 a
CC+EC+S (2:2:1)	4.44 a
V+EC (2:1)	4.44 a
CV (%)	11.71
	Length of the longest root
CC+EC (2:1)	6.23 ab
CC+EC+S (1:1:1)	6.04 b
CC+EC+S (2:1:1)	6.86 a
CC+EC+S (2:2:1)	6.30 ab
V+EC (2:1)	5.57 b
CV (%)	6.28
	Dry weight of aerial parts (g)
CC+EC (2:1)	120.55 a
CC+EC+S (1:1:1)	137.04 a
CC+EC+S (2:1:1)	117.36 a
CC+EC+S (2:2:1)	130.56 a
V+EC (2:1)	133.20 a
CV (%)	16.53
	Dry weight of roots (g)
CC+EC (2:1)	43.21 c
CC+EC+S (1:1:1)	130.16 a
CC+EC+S (2:1:1)	60.16 bc
CC+EC+S (2:2:1)	98.12 ab
V+EC (2:1)	87.28 b
CV (%)	24.25

CC = coconut coir; EC = earthworm castings; S = sand; V = vermiculite; CV = coefficient of variation. Same letters in the column do not differ according to Tukey's test ($p < 0.05$).

the variable length of the longest root, the substrates coconut coir : earthworm castings (2:1) and coconut coir : earthworm castings : sand (2:1:1 and 2:2:1) had the highest values (6.23, 6.86, and 6.30 cm), and the lowest average occurred when the substrate vermiculite : earthworm castings (2:1) was used. Possible reasons for this lower seedling development are the low content of organic matter present in vermiculite, the poor physical aggregation and possible nutrient limitation, and the low water retention in this substrate (ROCHA et al., 2009).

For the dry weight of roots, the substrates coconut coir: earthworm castings: sand (1:1:1 and 2:2:1) provided the best results (98.12 and 130.16 cm).

The coconut coir provides greater aggregation of roots to the substrate, facilitating the removal of seedlings from the trays and seedling development. This may be because coconut coir has physical and chemical characteristics suitable for the growth and development of macambira, which explains the successful development of the seedlings when this substrate was used. This substrate is also interesting because it is an abundant byproduct of the coconut agribusiness, available in great quantities in the northeast of Brazil and has a low market price, which would reduce the cost of production (ARRIGONI-BLANK et al., 2011). Lone et al. (2008) tested this substrate in the acclimatization of *Cattleya* seedlings (Orchidaceae) and was successful in this process.

Lédo et al. (2007) highlighted the importance of using coconut coir at the acclimatization stage because it gave the substrate a greater capacity to retain water and led to better survival and vigor of acclimated dwarf coconut seedlings.

4 Conclusions

The *in vitro* germination of *B. laciniosa* can be performed in a culture medium containing only water and agar. For acclimatization of *in vitro* macambira seedlings, the substrate coconut coir : earthworm castings : sand (2:2:1) should be used.

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