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In vitro multiplication of *Eugenia calycina* Cambess. (Myrtaceae) under the influence of BAP and NAA

Multiplicação *in vitro* de *Eugenia calycina* Cambess. (Myrtaceae) sob influência de BAP e ANA

Multiplicación *in vitro* de *Eugenia calycina* Cambess. (Myrtaceae) bajo la influencia de BAP y ANA

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Abstract
Eugenia calycina Cambess. (Myrtaceae) is a fruit species native to Brazil indicated for the recovery of degraded areas. Generally its propagation occurs by seeds, but leads to an increase in the juvenile period of the plants, problems in maintaining seed viability and pathogen incidence. However, with the application of tissue culture techniques it is possible to produce seedlings in large scale, uniformity of the plants obtained and reduction in the period of seed germination. Thus, the objective of this research was to define the best medium for *in vitro* multiplication of *E. calycina*. Nodal segments of 1 cm, extracted from *E. calycina* plants already established *in vitro*, were used as explants. The treatments consisted of different concentrations of BAP (0; 0.5; 1 and 2 mg L^{-1}) and NAA (0; 0.5; 1 and 2 mg L^{-1}). The experimental design was completely randomized, in a 4x4 factorial scheme, with four concentrations of BAP and four concentrations of NAA, with four replications and three tubes per replication. After 90 days, shoot number, length and fresh mass of the aerial part, production and fresh mass of callus were evaluated. The MS medium plus 2.0 mg L^{-1} of BAP is ideal for *in vitro* multiplication of *E. calycina*.
Keywords: Micropropagation; Growth regulators; Cytokinin; Auxin.

Resumo
Eugenia calycina Cambess. (Myrtaceae) é uma espécie frutífera nativa do Brasil indicada para recuperação de áreas degradadas. Geralmente sua propagação ocorre por sementes, porém acarreta em aumento do período juvenil das plantas, problemas na manutenção da viabilidade das sementes e incidência de patógenos. Contudo, com a aplicação de técnicas de cultura de tecidos é possível produzir mudas em larga escala, uniformização das plantas obtidas e redução no período de germinação das sementes. Sendo assim, o objetivo desta pesquisa foi definir o melhor meio para multiplicação *in vitro* de *E. calycina*. Segmentos nodais de 1 cm, extraídos de plantas de *E. calycina* já estabelecidas *in vitro*, foram utilizados como explantes. Os tratamentos foram constituídos de diferentes concentrações de BAP (0; 0,5; 1 and 2 mg L^{-1}) e ANA (0; 0,5; 1 e 2 mg L^{-1}). O delineamento experimental foi inteiramente casualizado, em esquema fatorial 4x4, sendo quatro concentrações de BAP e quatro concentrações de ANA, com quatro repetições e três tubos por repetição. Após 90 dias, foram avaliados número de brotos, comprimento e massa fresca da parte aérea, produção e massa fresca de calos. O meio MS acrescido de 2,0 mg L^{-1} de BAP é o ideal para multiplicação *in vitro* de plantas de *E. calycina*.
Palavras-chave: Micropropagação; Reguladores de crescimento; Citocinina; Auxina.

Resumen
Eugenia calycina Cambess. (Myrtaceae) es una especie frutal nativa de Brasil indicada para la recuperación de áreas degradadas. Generalmente su propagación es por semillas, pero esto conlleva un aumento del período juvenil de las plantas, problemas en el mantenimiento de la viabilidad de las semillas y la incidencia de patógenos. Sin embargo,

con la aplicación de técnicas de cultivo de tejidos es posible producir plántulas en gran escala, uniformidad de las plantas obtenidas y reducción del período de germinación de las semillas. Así, el objetivo de esta investigación fue definir el mejor medio para la multiplicación *in vitro* de *E. calycina*. Se utilizaron como explantes segmentos nodales de 1 cm, extraídos de plantas de *E. calycina* ya establecidas *in vitro*. Los tratamientos consistieron en diferentes concentraciones de BAP (0; 0,5; 1 y 2 mg L^{-1}) y ANA (0; 0,5; 1 y 2 mg L^{-1}). El diseño experimental fue completamente al azar, en esquema factorial 4x4, con cuatro concentraciones de BAP y cuatro concentraciones de NAA, con cuatro repeticiones y tres tubos por repetición. Después de 90 días, se evaluaron el número de brotes, la longitud y la masa fresca de la parte aérea, la producción y la masa fresca de callo. El medio MS más 2,0 mg L^{-1} de BAP es ideal para la multiplicación *in vitro* de *E. calycina*.
Palabras clave: Micropropagación; Reguladores del crecimiento; Citoquinina; Auxina.

1. Introduction

The Cerrado is one of the largest Brazilian biomes and with the presence of a large amount of native fruit trees. However, some species have difficulty propagating via seeds, as they have heterogeneity in the maturation process of fruits and seeds with some type of dormancy. Factors that can harm the production of seedlings on a commercial scale (Pinhal et al., 2011).

Eugenia calycina Cambess., known as pitanga vermelha or pitanga do Cerrado, belongs to the Myrtaceae family and comprises approximately 100 genera and 3,500 species, which are found mainly in tropical and subtropical regions of the world. This species can occur in the shrub form, commonly found in the Cerrado, or in the arboreal or subarboreal form, which is typical of riparian forests. Its propagation can be done by seeds, grafting and cutting methods. The production of seedlings from seeds is the most used because the species has good germination condition. However, there are problems regarding seed storage of the species due to low longevity and high incidence of pathogens (Von Bulow et al., 1994).

Tissue culture is characterized by the *in vitro* cultivation of plants from cells, tissues, organs, embryos or fragments of living tissues in a nutrient medium under aseptic conditions and a controlled environment. In addition, this biotechnological tool is of great importance in plant genetic improvement, germplasm conservation, industrial propagation and plant conservation, as well as research in plant physiology and *in vitro* production of secondary compounds (Carvalho et al., 2003).

Thus, with the application of tissue culture techniques to fruit trees in the Cerrado, it becomes possible to solve or minimize some of the problems regarding the propagation of these species; through the systematic multiplication of plants, exchange of genetic material, reduction in the germination period, germplasm maintenance, standardization of the plants obtained, among other techniques (Melo et al., 2002).

Therefore, the objective of this research to define the best medium for *in vitro* multiplication of *E. calycina* through the use of BAP and NAA.

2. Methodology

The experiment was carried out at the Plant Tissue Culture Laboratory of the Department of Agriculture (DAG) of the Federal University of Lavras (UFLA), Lavras-MG, Brazil.

Nodal segments of 1 cm in length, extracted from *Eugenia calycina* plants already established *in vitro*, were used as explants.

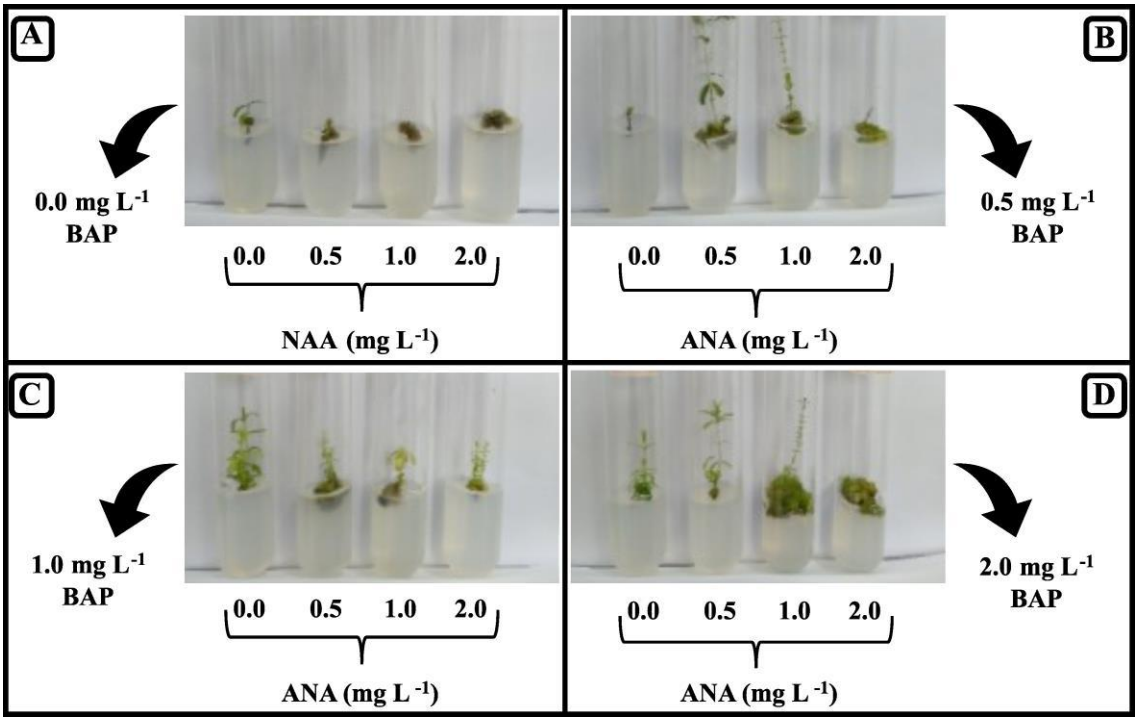
The culture medium used in the experiment was MS (Murashige & Skoog, 1962) containing 30 g L^{-1} sucrose, 5.5 g L^{-1} agar and pH 6.0. The treatments consisted of concentrations of BAP (0; 0.5; 1 and 2 mg L^{-1}) and NAA (0; 0.5; 1 and 2 mg L^{-1}) in possible combinations. The culture medium was distributed in test tubes (25 mm x 150 mm) containing 15 mL of medium per tube, and autoclaved at 121ºC and 1.0 atm pressure for 20 min. After autoclaving, the tubes were taken to a laminar flow chamber, where the nodal segments were inoculated. The tubes were kept in a growth room at a temperature of 25 ± 1 °C, irradiance of 47.6 µmol m^{-2} s^{-1} and photoperiod of 16 h.

The experimental design was completely randomized, in a 4x4 factorial scheme, with four concentrations of BAP and four concentrations of NAA, with four replications and three tubes per repetition. After 90 days, the number of shoots, shoot length (cm), shoot fresh mass (g), production and calli fresh mass (g) were evaluated. Data were subjected to analysis of variance and, when significant, regression analysis was performed at a 5% probability level, using the statistical program SISVAR (Ferreira, 2011).

3. Results and Discussion

Many researchers have already worked on the use of BAP aiming at the *in vitro* multiplication of various species (Arruda et. al., 2011; Asmar et al., 2019; Salem et al., 2022; Villa et al., 2005; Villa et al., 2006). From the present study, it was possible to establish the most suitable medium for *in vitro* multiplication of *Eugenia calycina*, and thus, in Figure 1 it is possible to observe the behavior of the species in different treatments containing the growth regulators BAP and NAA.

Figure 1 - *In vitro* multiplication of *E. calycina* under the influence of BAP and NAA.

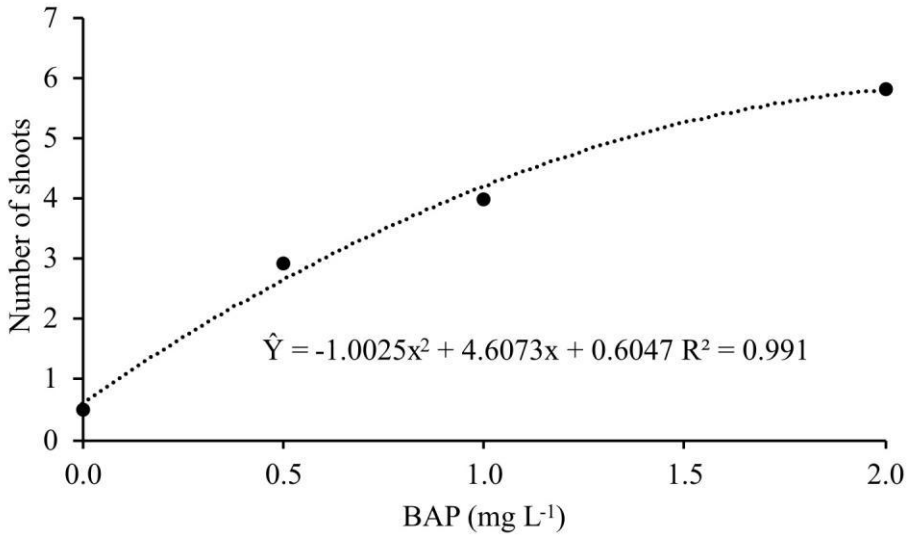


A) 0.0 mg L^{-1} BAP + 0.0; 0.5; 1.0 and 2.0 mg L^{-1} NAA; B) 0.5 mg L^{-1} BAP + 0.0; 0.5; 1.0 and 2.0 mg L^{-1} NAA; C) 1.0 mg L^{-1} BAP + 0.0; 0.5; 1.0 and 2.0 mg L^{-1} NAA; and D) 2.0 mg L^{-1} BAP + 0.0; 0.5; 1.0 and 2.0 mg L^{-1} NAA. Fonte: Filipe Almendagna Rodrigues.

Significant interaction was observed in the association between BAP and NAA factors for shoot length, callus number and fresh mass of callus of *E. calycina*. However, a significant effect was observed for the number of shoots and fresh mass of shoots between the factors alone (BAP and NAA).

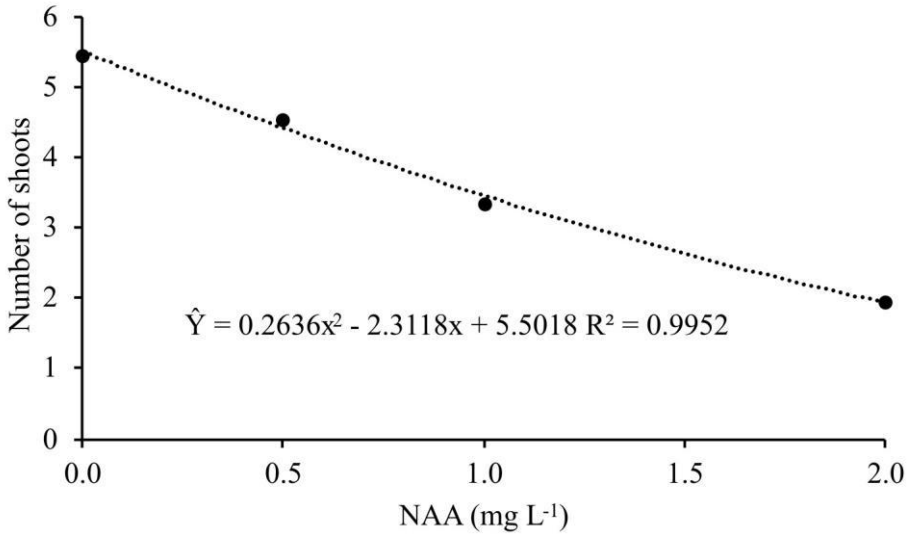
BAP provided a greater increase in the number of shoots as its concentration increased, whereas NAA contributed to a reduction in the number of shoots with the increase in its concentration in the culture medium (Figure 2). While the highest number of shoots (5.85) was observed at the concentration of 2.0 mg L^{-1} BAP in the absence of NAA (Figure 3). Nascimento et al. (2008) studied the effect of the addition of BAP in cauline segments of uvaieira (*E. pyriformis* Cambess) and did not observe significant differences for the number of shoots when the explants were submitted to different concentrations of BAP.

Figure 2 - Number of shoots from explants of *E. calycina* under the influence of BAP.



Fonte: Filipe Almendagna Rodrigues.

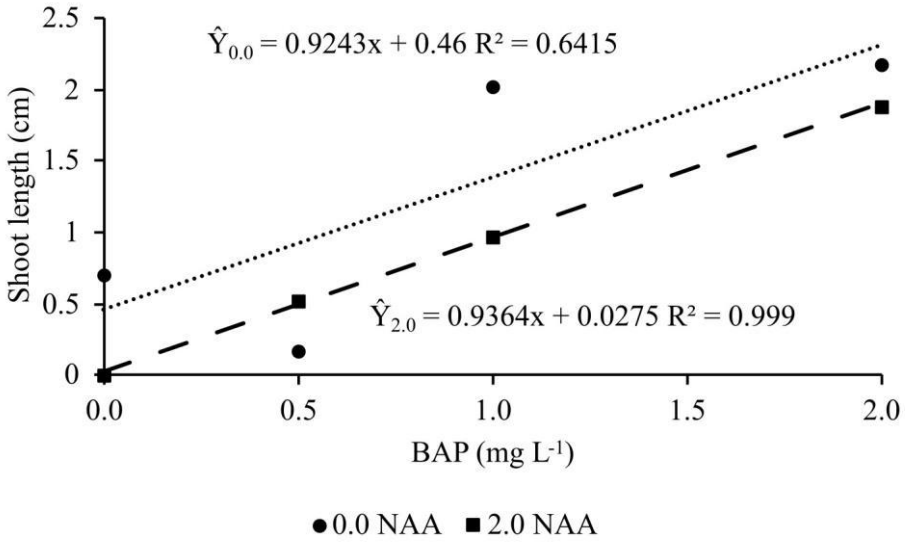
Figure 3 - Number of shoots from explants of *E. calycina* under the influence of NAA.



Fonte: Filipe Almendagna Rodrigues.

Greater shoot lengths were obtained, respectively, in the combination of 2.0 mg L^{-1} BAP and 2.0 mg L^{-1} NAA (1.88 cm) or 2.0 mg L^{-1} BAP in the absence of NAA (2.17 cm) (Figure 4). Furthermore, higher concentrations of BAP provided greater shoot lengths, regardless of the concentration of NAA used (0 or 2.0 mg L^{-1} NAA).

Figure 4 - Shoot length of *E. calycina* explants under the influence of BAP and NAA.

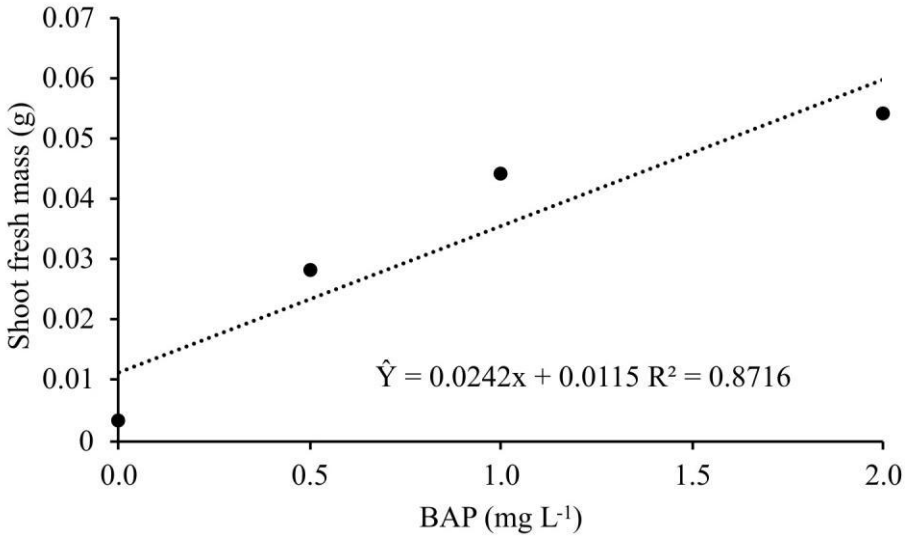


Fonte: Filipe Almendagna Rodrigues.

Rodrigues et al. (2013) also induced *in vitro* sprouting in *Physalis peruviana* L. and found that BAP concentrations above 1.0 mg L^{-1} were efficient for the *in vitro* multiplication of the species. Furthermore, Souza et al. (2008) evaluated the effect of different concentrations of cytokinin on the *in vitro* multiplication of *E. uniflora* L. and concluded that the lower concentration of BAP promoted satisfactory results, in addition to allowing cost reduction. Villa et al. (2009) multiplied grapevine rootstocks *in vitro* using different concentrations of adenine sulfate and BAP. Therefore, better results were obtained in culture media containing 1.0 mg L^{-1} BAP.

A significant effect was observed between BAP concentrations for shoot fresh mass, with the highest increase (0.0543 g) occurring at the BAP concentration of 2.0 mg L^{-1} (Figure 5).

Figure 5 - Shoot fresh mass of *E. calycina* explants under the influence of BAP.

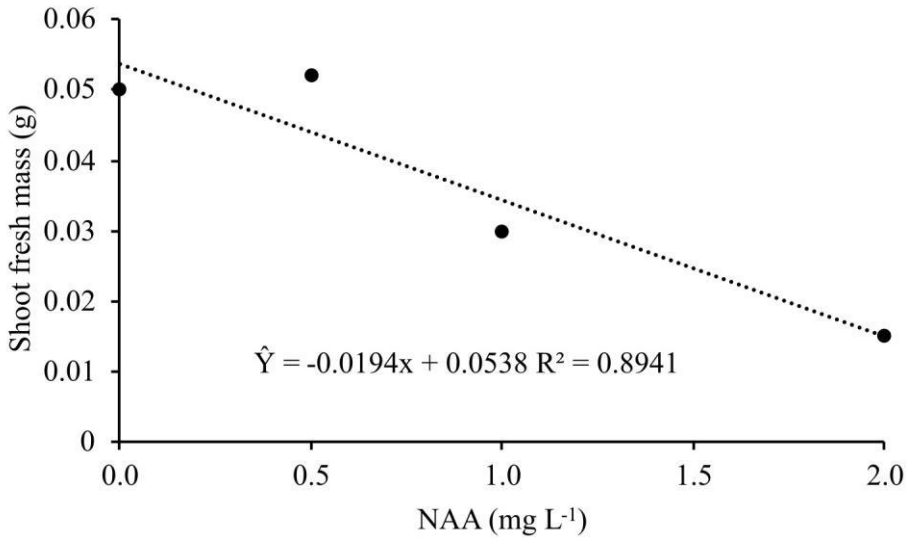


Fonte: Filipe Almendagna Rodrigues.

However, when evaluating the effect of NAA, it was verified that the 2.0 mg L^{-1} concentration promoted a smaller

increase in mass (0.0152 g) (Figure 6). In the control treatment, in which BAP was not used, the shoot fresh mass was 0.003 g, while in the 2.0 mg L^{-1} concentration it reached 0.0543 g (Figure 6).

Figure 6 - Shoot fresh mass of *E. calycina* explants under the influence of NAA.

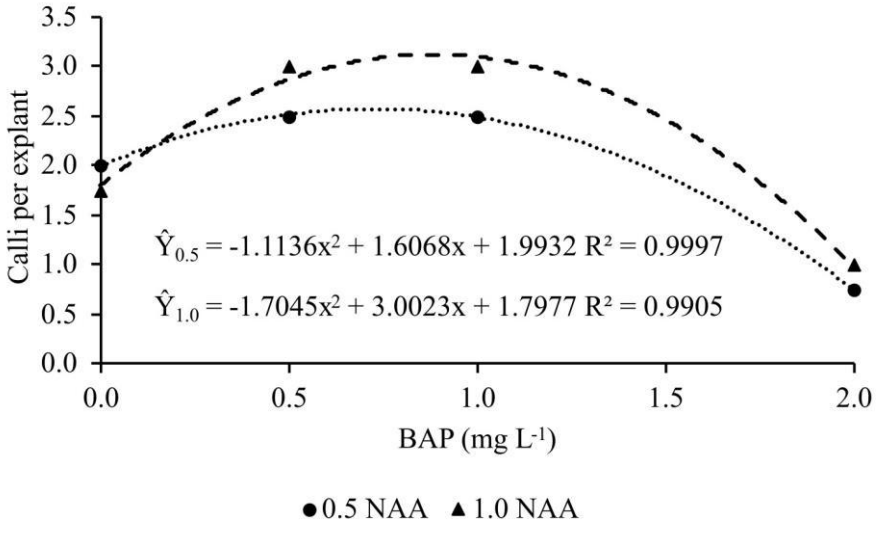


Fonte: Filipe Almendagna Rodrigues.

Asmar et al. (2012) tested different concentrations of BAP for the *in vitro* multiplication of *Lippia alba*, and concluded that 1.5 mg L^{-1} BAP was the most favorable concentration for the multiplication of this species. However, the absence of BAP provided a greater number of leaves. The concentration 0.5 mg L^{-1} BAP increased the fresh and dry mass of shoots in this species.

Greater callus production by explants occurred in the significant interaction between BAP and NAA factors (Figure 7). In the combinations of 1.0 mg L^{-1} BAP and 0.5 mg L^{-1} NAA and 1.0 mg L^{-1} BAP and 1.0 mg L^{-1} NAA, respectively, 2.5 and 3.0 calli per explant.

Figure 7 - Calli per explant from *E. calycina* under the influence of BAP and NAA.

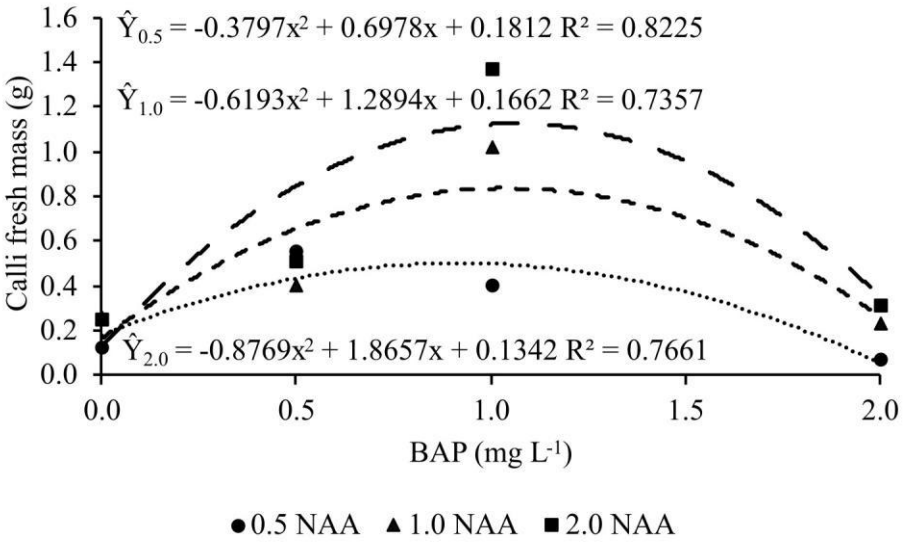


Fonte: Filipe Almendagna Rodrigues.

Bordignon et al. (2022) evaluated the presence and absence of callus in the explants, and were able to observe significant differences between treatments supplemented with BAP and NAA related to the production of callus by the explants. Thus, the researchers observed that the greatest callus production occurred in media containing 0.22; 0.33 and 0.44 mg L^{-1} BAP and 0.1 mg L^{-1} NAA.

Significant interaction was observed between BAP and NAA factors for calli fresh mass (Figure 8). Greater increases were observed in the combinations of 0.5 mg L^{-1} BAP and 0.5 mg L^{-1} NAA (0.5632 g); 1.0 mg L^{-1} BAP and 1.0 mg L^{-1} NAA (1.0245 g) and 1.0 mg L^{-1} BAP and 2.0 mg L^{-1} NAA (1.3698 g).

Figure 8 - Calli fresh mass from explants of *E. calycina* under the influence of BAP and NAA.

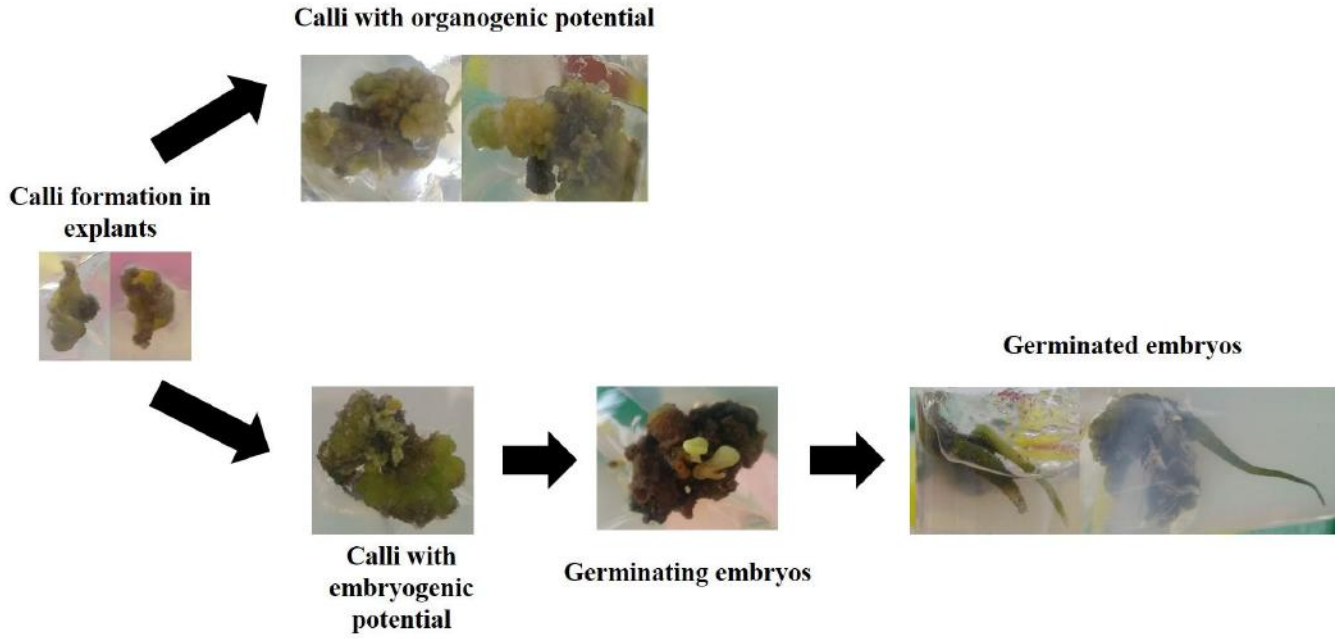


Fonte: Filipe Almendagna Rodrigues.

Nascimento et al. (2008) observed significant differences in the average number of leaves in segments of uvaieira (*E. pyriformis* Cambess.) in the different concentrations of BAP tested, however the highest average referring to the number of leaves was also found in the treatment supplemented with BAP, at a concentration of 1.0 mg L^{-1}.

Furthermore, it is worth mentioning that the researchers in the present study have been carrying out research with the species *E. calycina* aiming to obtain plants using *in vitro* indirect organogenesis and somatic embryogenesis techniques (Figure 9). Through this technique, it will be possible to maintain the genetic characteristics of the species, as well as prevent its extinction process from occurring.

Figure 9 - Organogenesis and indirect somatic embryogenesis techniques in *E. calycina* plants.



Fonte: Filipe Almendagna Rodrigues.

4. Conclusion

The MS medium added with 2.0 mg L^{-1} BAP is ideal for the *in vitro* multiplication of *Eugenia calycina* plants.

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