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In vitro germination of babassu: influence of growth regulators in zygotic embryos

Mariluza Silva Leite[1], Fabiano Guimarães Silva[1*], Flávia Dionísio Pereira[2], Paula Sperotto Alberto[1] and Alessandra Cristina Boffino de Almeida Monteiro-Hara[1]

*[1]Instituto Federal Goiano, Campus Rio Verde, Rodovia Sul Goiana, Km 1, 75901-970, Rio Verde, Goiás, Brazil. [2]Cooperativa Agroindustrial dos Produtores Rurais do Sudoeste Goiano Ltda., Rio Verde, Goiás, Brazil. *Author for correspondence. E-mail: fabianoifgoiano@gmail.com*

ABSTRACT. Babassu is a highly productive oil plant that is propagated from seed. Therefore, tissue culture techniques are of great importance because propagation is accelerated. The objective of this study was to evaluate the effect of growth regulators, NAA, BAP, and KIN, on *in vitro* germination and on the growth of babassu zygotic embryos. Three trials were conducted. For trial (I), the interaction between NAA (0, 1, and 2 μM) and KIN (0, 1, and 2 μM) were studied; for trial (II), the interactions among NAA (0, 1, and 2 μM), KIN (0, 1, and 2 μM), and BAP (1 μM) were studied; and for trial (III), the interactions among NAA (0, 1, and 2 μM), KIN (0, 1, and 2 μM), and BAP (2 μM) were studied. At 30, 60, 90, and 120 days of culture, we assessed average seedling height, and at 120 days, we assessed germination, the germination speed index, cotyledonary petiole growth, oxidation, and the formation of shoots, roots, and calluses. The best results for cotyledonary petiole formation and average seedling height were obtained when using 1 μM of BAP in the culture medium. We also found that the 2 μM of NAA favored root formation.

Keywords: *Orbignya oleifera* (Burret.), biodiesel, Arecaceae.

Germinação *in vitro* de babaçu: influência de reguladores de crescimento em embriões zigóticos

RESUMO. O babaçu é uma palmeira oleaginosa, que se propaga via sementes. A contribuição das técnicas de cultura de tecidos é de grande importância, pois acelera sua propagação. Objetivou-se com este trabalho avaliar o efeito dos reguladores NAA, BAP e KIN na germinação e no crescimento *in vitro* de embriões zigóticos de babaçu. Para isso foram feitos três ensaios: Ensaio (I) interação de NAA (0, 1 e 2 μM) e KIN (0, 1 e 2 μM); Ensaio (II) interação de NAA (0, 1 e 2 μM), KIN (0, 1 e 2 μM) e BAP (1 μM); Ensaio (III) interação de NAA (0, 1 e 2 μM), KIN (0, 1 e 2 μM) e BAP (2 μM). Aos 30, 60, 90 e 120 dias de cultivo, avaliou-se o comprimento médio das plântulas e aos 120 dias germinação, índice de velocidade de germinação, crescimento do pecíolo cotiledonar, formação de parte aérea, raiz, calos e oxidação. Verificou-se que os melhores resultados para formação do pecíolo cotiledonar e o comprimento médio de plântulas foram obtidos com a utilização de 1 μM de BAP no meio de cultivo, e que a concentração de 2 μM de NAA favoreceu a formação de raízes.

Palavras-chave: *Orbignya oleifera* (Burret.), biodiesel, Arecaceae.

Introduction

Babassu, (*Orbignya oleifera* Burret.), which belongs to the Arecaceae family, is a large palm that can reach up to 20 min. in height with a 41 cm stipe diameter and a leaf length up to 8 m. Popularly known as babassu, bauaçu, baguaçu, auaçu, and monkey-coconut, the height of its fruit yield occurs between August and January, with 2,000 fruit per year (LIMA et al., 2007).

Babassu has a high consumption potential, and it contributes significantly to the economies of many Brazilian federal states, especially Acre, Maranhão, Tocantins, and Goiás, where the well-known massifs are located. The main commercial products extracted from the babassu palm include oil (extracted from the seed) and cake (residue from extracting oil from the seed). The fruit provides a vegetable butter with a pleasant taste and nutritional value. The fruit mesocarp produces an excellent quality charcoal, which is used as an energy source in steelworks. Thus, babassu is considered a primary alternative among Brazilian oilseeds for biofuel production. The babassu seed contains between 60-70% oil; however, it only represents 6-10% of the fruit fresh weight. The nuts can be eaten raw or processed to obtain its lauric acid-rich oil, which is used in human food, cosmetics, and lubricants, or it

can be transformed into biodiesel (LIMA et al., 2006; TEIXEIRA, 2005).

Biodiesel production in Brazil has a favorable outlook, which is an advantage of this country in relation to other oilseed producers. However, production is still incipient, considering the potential it has with respect to its territorial dimensions primarily in Brazilian Cerrado (savannah) regions, its high edaphoclimatic diversity, and the great number of plant species that can be used for this purpose. When considering biodiesel production, it appears that the seeds and seedlings of species with a high productive potential for biodiesel production become an extremely scarce commodity. Furthermore, one of the main challenges for agricultural research is producing improved cultivars with genetic stability, high quality, and productive potential. Therefore, we see the need for increased actions aimed towards the constant modernization of industrial parks and expanding internal and external consumption.

Introducing the commercial cultivation of palms is a difficult task due to pronounced seed dormancy, which with a high oil content is also susceptible to decay (BEWLEY; BLACK, 1994; HIANE et al., 2005; MARCOS FILHO, 2005; TEIXEIRA, 2005). Excellent results have been obtained in experiments using current technological advances involving *in vitro* culture techniques (PECH-AKÉ et al., 2007; PEREIRA et al., 2006; SARASAN et al., 2002; SILVA et al., 2012). These new techniques were adopted to overcome propagation problems. Thus, using in vitro culture techniques became important for the production of healthy and well-developed seedlings, making it an essential factor for the success of new crops.

In vitro culture is used when sexual propagation is unsatisfactory, i.e., when the progeny obtained is very heterogeneous or when propagation by seed does not occur naturally. Zygotic embryo culture is a viable alternative for various palm species and offers the opportunity to expand studies on germination. This technique has been used to overcome dormancy in commercial-scale production to obtain high-quality and healthy plants and to determine the nutritional and physical needs in seedling development (MELO et al., 2001a; MOLLA et al., 2004; SPERA et al., 2001; TZEC-SIMA et al., 2006).

However, commercial-scale reproduction requires an improved understanding of the factors that control *in vitro* morphogenesis and the factors that limit the rates of reproduction and rooting. Conventional systems for inducing morphogenetic responses can be improved by manipulating the determining factors *in vitro*. Various factors can influence the regenerative potential of a species, and it is necessary to select the appropriate culture medium, including whether or not growth regulators are added, which is a choice that completely depends on the objective, as the outcomes will differ. Specific trials are essential to establish an ideal protocol with effective combinations and concentrations to induce the expected response (LEDO et al., 2007; MELO et al., 2001b; TZEC-SIMA et al., 2006).

Despite the economic importance of *Orbignya oleifera* (Burret.) in the Brazilian region, there are no reports on the *in vitro* culture of this species. Therefore, the objective of the current study was to assess the effect of the auxin/cytokinin interaction on germination and seedling growth in zygotic embryos.

Material and methods

Obtaining plant material

The trials were conducted at the Laboratório de Cultura de Tecidos Vegetais do Instituto Federal Goiano, Campus Rio Verde, Goiás State, Brazil.

The fruit were collected after abscission in January 2011 from a population of plants at the Santa Bárbara farm in the municipality of Piranhas, Goiás State, Brazil. The coordinates of the location are 16º 22.015' S 51º 55.715' W, 389 m altitude (Figure 1A). The voucher specimen is deposited in the RBR herbarium at the Federal University of Goiás under collection no 5641.

After sampling, the ripe babassu fruit were crushed in a hydraulic press to break the endocarp, the protective tissue that makes the fruit rigid (Figure 1B). Later, the nuts were taken from inside the fruit (Figures 1C and D), and the embryos were removed from the nuts (Figure 1E).

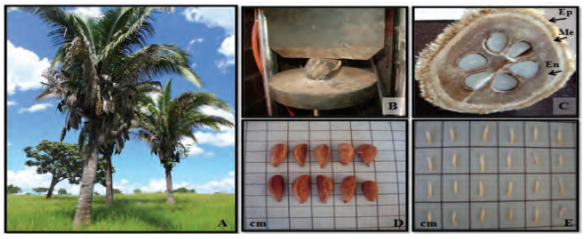

Figure 1. Highlighting the plant matrix of *Orbignya oleifera* (Burret.) at the Santa Bárbara farm in the municipality of Piranhas, GO, Brazil (A); hydraulic press used to extract the seeds (B); a cross section of a babassu fruit (C); seeds (D); and babassu zygotic embryos (E). Ep: epicarp; Me: mesocarp; and En: endocarp. Scale: 2 cm. Photo: Mariluza S. Leite. Rio Verde, Goiás State, Brazil, 2011.

Asepsis

In all of the trials, the embryos were covered with gauze and immersed in 70% ethanol for 1 min.; the embryos were then immersed in a 20% sodium hypochlorite solution (NaOCl; commercial bleach, 2.5% active chlorine) for 20 min. and washed 3 times with sterile water in a laminar flow chamber.

In vitro establishment

The babassu embryos were inoculated in test tubes (25 x 150 mm) containing 20 mL of MS medium at 50% salt concentration (MURASHIGE; SKOOG, 1962) supplemented with 3.5 g L^{-1} of agar (Dinâmica®).

Naphthaleneacetic acid (NAA), 6-benzylaminopurine (BAP), and kinetin (KIN), at different concentrations, were added to the culture medium, thus establishing the 3 trials described below:

Trial (I): NAA x KIN interaction

The inoculated embryos in this trial were arranged in MS medium, in which 9 combinations of NAA and KIN were tested (μM): (T_1) 0 NAA + 0 KIN, (T_2) 0 NAA + 1 KIN, (T_3) 0 NAA + 2 KIN, (T_4) 1 NAA + 0 KIN, (T_5) 1 NAA + 1 KIN, (T_6) 1 NAA + 2 KIN, (T_7) 2 NAA + 0 KIN, (T_8) 2 NAA + 1 KIN, and (T_9) 2 NAA + 2 KIN.

Trial (II): NAA x KIN x (1 μM) BAP interaction

The embryos were inoculated in 50% MS medium containing the following combinations of NAA, KIN, and BAP (μM): (T_1) 0 NAA + 0 KIN + 1 BAP, (T_2) 0 NAA + 1 KIN + 1 BAP, (T_3) 0 NAA + 2 KIN + 1 BAP, (T_4) 1 NAA + 0 KIN + 1 BAP, (T_5) 1 NAA + 1 KIN + 1 BAP, (T_6) 1 NAA + 2 KIN + 1 BAP, (T_7) 2 NAA + 0 KIN + 1 BAP, (T_8) 2 NAA + 1 KIN + 1 BAP, and (T_9) 2 NAA + 2 KIN + 1 BAP.

Trial (III): NAA x KIN x (2 μM) BAP interaction

The embryos were inoculated in 50% MS medium containing the following combinations of NAA, KIN, and BAP (μM): (T_1) 0 NAA + 0 KIN + 2 BAP, (T_2) 0 NAA + 1 KIN + 2 BAP, (T_3) 0 NAA + 2 KIN + 2 BAP, (T_4) 1 NAA + 0 KIN + 2 BAP, (T_5) 1 NAA + 1 KIN + 2 BAP, (T_6) 1 NAA + 2 KIN + 2 BAP, (T_7) 2 NAA + 0 KIN + 2 BAP, (T_8) 2 NAA + 1 KIN + 2 BAP, and (T_9) 2 NAA + 2 KIN + 2 BAP.

The pH was adjusted to 5.7 ± 3 before autoclaving at 121°C at a pressure of 1.05 kg cm^{-2} for 20 min.

Following inoculation, the tubes containing the embryos were maintained in a growth room at 16-hours photoperiod for 120 days under the following conditions: a temperature of 25±3°C, 45% relative humidity, and photosynthetic active radiation of 45-55 μmol m^{-2} s^{-1}.

Evaluation and Experimental Design

All trials were evaluated on alternate days to determine the germination percentage and the germination speed index (GSI). At 30, 60, 90, and 120 days of culture, the average seedling height was assessed, and at 120 days, cotyledonary petiole growth, oxidation, and the formation of shoots, roots, and callus were assessed.

The 3 experimental trials had completely randomized designs (CRD) in a factorial arrangement: 9 treatments with 25 replicates (consisting of 1 test tube each). The data were statistically analyzed using analysis of variance, and means testing was performed using the Scott-Knott test. The percentage data were transformed into arcsin √% and number of counts in √x+1. The SISVAR software was used for the data analysis (FERREIRA, 2011).

Results and discussion

Trial (I): Interaction between NAA and KIN during in vitro germination and growth of babassu zygotic embryos

The babassu (*Orbignya oleifera* Burret) seed consists of a tegument, an endosperm, and an embryo. It is elongated, approximately 4.5 cm long, and has one straight side and one curved side, a structure that is adequate to accommodate the embryo. The width of the median region is approximately 1.0 cm (Figure 2A).

The tegument is brown colored and has numerous invaginations caused by friction between the endocarp and endosperm (Figure 2A). The embryo is laterally inserted at the end of the seed and consists of a cylindrical region, which is the cotyledonary petiole, and a distal region that makes up the cotyledonary limb or the haustorium (Figure 2B).

The structure of the babassu embryo has a general pattern that is observed in other palms; peculiarly, it is bulge-shaped with a haustorium with pronounced invaginations (HENDERSON, 2006; PANZA et al., 2004). However, according to Aguiar and Mendonça (2003) and Orozco-Segovia et al. (2003), palm seeds generally contain small embryos in relation to seed size and a high amount of endosperm. In the case of babassu, the embryos are larger than the standard size that is observed in palms, averaging 1 cm long and 0.1 cm diameter.

In vitro reproduction of babassu via zygotic embryos is acceptably viable, as all treatments showed germination within the first 7 days of culture. During this period, imbibition of the embryos was visible, as indicated by the expanding cotyledonary petiole, which is located in the basal region. This process was followed by a reduction of the haustorium, which atrophies and tends to detach from the cotyledonary petiole (Figure 2C).

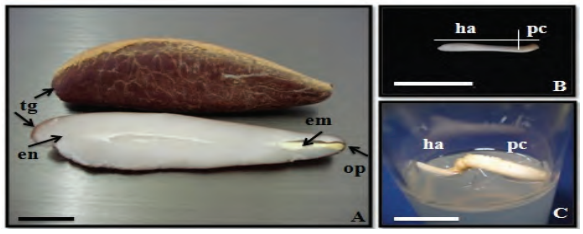

Figure 2. *Orbignya oleifera* (Burret.) seeds, whole and a cross-section (A); zygotic embryo (B); germinated embryo (C). (Bar equal to 1.0 cm). em: embryo; tg: tegument; en: endosperm; op: operculum; pc: petiole; ha: haustorium. Photo: Mariluza S. Leite. Rio Verde, Goiás State, Brazil (2011).

Next, the percentage of germinated embryos was quantified. According to an analysis of variance and the Scott-Knott test, the following characteristics were not affected: the germination speed index (SPI), the formation of shoots, oxidation, and the average seedling growth.

In relation to the other characteristics assessed, germination and cotyledonary petiole, root, and callus formation, the NAA x KIN interaction had no effect. In addition, the use of KIN did not affect the following characteristics: cotyledonary petiole, root, and callus formation. The use of NAA had no significant effect on germination (Table 1).

The highest average germination percentages, 80% and 88%, were obtained using a culture medium without KIN and at 1 µM, respectively. The lowest average percentage, 71%, was found when using 2 µM of KIN.

The highest average rate of cotyledonary petiole formation, 28%, was obtained without the use of NAA. The lowest averages of 5.3% and 1.3% were obtained with the media containing 1 and 2 µM of NAA, respectively (Table 1).

As for the roots, unlike the cotyledonary petiole, culture medium without NAA provided a lower average percentage of root formation, 1.3%. The highest average percentages of 21.3% and 20.0% were obtained for media with 1 and 2 µM of NAA, respectively. The presence of NAA improved root formation, while its absence reduced root development (Table 1).

Table 1. The germination percentage and the formation of the cotyledonary petiole, roots, and calluses in babassu (*Orbignya oleifera* Burret) zygotic embryos at 120 days of culture for various combinations of NAA and KIN. Rio Verde, Goiás State, Brazil, 2011.

NAA (µM)	KIN (µM)			
	0	1	2	
	Germination (%) at 120 days			Mean
0	72.0 Aa[Z]	92.0 Aa	72.0 Aa	79.0 A
1	88.0 Aa	88.0 Aa	64.0 Ab	80.0 A
2	80.0 Aa	84.0 Aa	76.0 Aa	80.0 A
Mean	80.0 a	88.0 a	71.0 b	
	Cotyledonary petiole (%) at 120 days			Mean
0	8.0 Ab	44.0 Aa	32.0 Aa	28.0 A
1	8.0 Aa	8.0 Ba	0.0 Ba	5.3 B
2	4.0 Aa	0.0 Ba	0.0 Ba	1.3 B
Mean	6.7 a	17.3 a	10.7 a	
	Root formation (%) at 120 days			Mean
0	0.0 Ba	4.0 Aa	0.0 Ba	1.3 B
1	32.0 Aa	20.0 Aa	12.0 Aa	21.3 A
2	32.0 Aa	12.0 Aa	16.0 Aa	20.0 A
Mean	21.3 a	12.0 a	9.3 a	
	Callus formation (%) at 120 days			Mean
0	0.0 Ba	0.0 Ba	0.0 Ba	0.0 C
1	36.0 Aa	12.0 Bb	12.0 Bb	20.0 B
2	28.0 Aa	36.0 Aa	36.0 Aa	33.3 A
Mean	21.3 a	16.0 a	16.0 a	

[Z]Means followed by the same uppercase letter in each column and the same lowercase letter in each row do not differ by a Scott-Knott test at 5% probability.

For callus formation, the highest NAA concentrations provided the highest growth rates. When using 2 and 1 µM of NAA, average rates of 33.3% and 20% were obtained, respectively, whereas no callus formation occurred without the use of NAA. The combination of NAA x KIN did not have a significant effect on the growth rate, when 1 µM NAA was used with increasing concentrations of KIN, callus formation was reduced, which represents a favorable result (Table 1).

Thus, roots and calluses were formed when using NAA, whereas there was a reduction in cotyledon petiole formation. To optimize the specific growth regulators to include and their concentration, BAP (1 and 2 µM) was added to medium in trials II and III. The results are shown in Tables 2 and 3.

Accompanying the statistical data, visual observations included the following: cream-colored embryos grew with an elongating cotyledonary petiole that remained the same color, from which green colored shoots (cotyledonary leaves) developed (Figure 3). More petiole growth (Figure 3A, B and C) and less root formation (Figure 3D, E and F) were observed for treatments that used culture medium without NAA and/or 1 µM of NAA. High NAA concentrations showed a trend of callus formation and an appearance of multiple roots. Calluses that formed from the petiole base were frail and brown (Figure 3G, H and I).

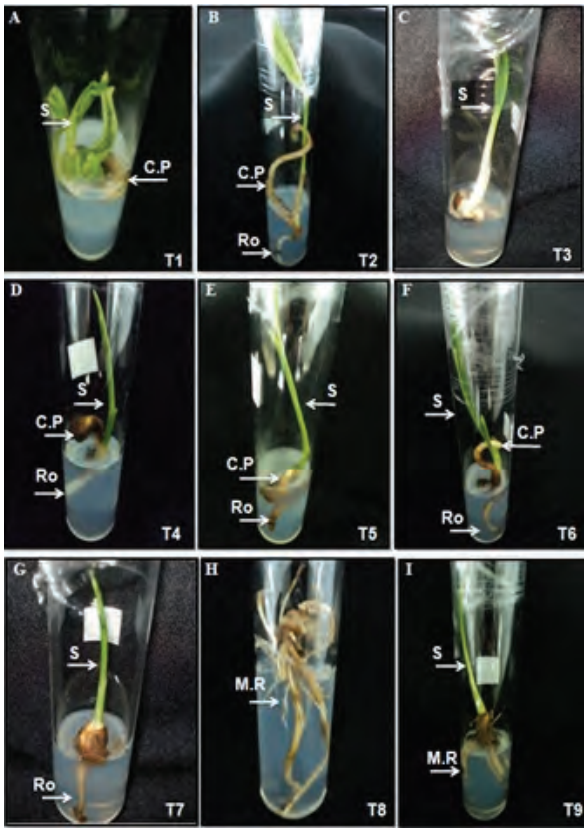

Figure 3. *In vitro* germination and growth of babassu (*Orbignya oleifera* Burret) zygotic embryos cultured for 120 days in 50% MS medium supplemented with various concentrations of NAA and KIN: A) T1: NAA (0 µM) and KIN (0 µM); B) T2: NAA (0 µM) and KIN (1 µM); C) T3: NAA (0 µM) and KIN (2 µM); D) T4: NAA (1 µM) and KIN (0 µM); E) T5: NAA (1 µM) and KIN (1 µM); F) T6: NAA (1 µM) and KIN (2 µM); G) T7: NAA (2 µM) and KIN (0 µM); H) T8: NAA (2 µM) and KIN (1 µM) and I) T9: NAA (2 µM) and KIN (2 µM). Shoot (S); cotyledonary petiole (C.P); root (Ro); multiple roots (M.R).

Trial (II): Interaction between NAA and KIN in culture medium supplemented with BAP (1 μM) during in vitro germination and growth of babassu (Orbignya oleifera Burret.) zygotic embryos

Among the assessed characteristics, the following remained unaffected: germination, the germination speed index (GSI), shoot formation, oxidation (assessed at 120 days of culture), and the average seedling height (assessed at 90 and 120 days of culture) (Table 2).

There was a significant interaction between NAA x KIN in terms of callus formation. The highest NAA concentrations that were used provided the highest growth rates. When using 2 or 1 µM of NAA, average rates of 38.67% and 20.0% were obtained, respectively; no callus formation occurred without NAA (Table 2). Regarding the KIN growth regulator, a lower average percentage, 9.33%, of callus formation occurred when using 2 µM. The highest average percentages were obtained in culture medium without KIN or with 1 µM of KIN, 25.33% and 24.0%, respectively. Thus, the addition of NAA increases callus formation. However, the combination of this regulator with higher doses of KIN reduces this characteristic. This combination is recommended to decrease the callus formation, which would be undesirable for this case (Table 2).

There was no significant effect of either the combination of NAA x KIN or the use of KIN alone on the other characteristics that were assessed: cotyledonary petiole formation, root formation, and the average seedling height (assessed at 30 and 60 days of culture) (Table 2).

Adding NAA to culture medium did not enhance the average seedling height, neither for those assessed at 30 days nor for those at 60 days. The largest average seedlings were obtained in medium without NAA, 2.28 cm and 3.21 cm, assessed at 30 days and 60 days of culture, respectively (Table 2).

Adding NAA to culture medium also did not enhance average cotyledonary petiole formation, obtaining a percentage of formation of 50.67% in medium without NAA and values of 9.33% and 4.0% when adding 1 and 2 µM of NAA, respectively (Table 2).

Table 2. Average seedling height, which was evaluated at 30 and 60 days of culture, and the formation of cotyledonary petioles, roots, and calluses, which were evaluated at 120 days of culture in babassu (*Orbignya oleifera* Burret.) zygotic embryos. The embryos were cultured *in vitro* with various concentrations of NAA and KIN, supplemented with 1 μM of BAP. Rio Verde, Goiás State, Brazil, 2011.

NAA (μM)	KIN (μM) 0	1	2	
	Average seedling height (cm) at 30 days			Mean
0	1.98 Aa[z]	2.68 Aa	2.17 Aa	2.28 A
1	1.84 Aa	1.58 Ba	1.83 Aa	1.75 B
2	1.52 Aa	1.23 Ba	1.62 Aa	1.46 B
Mean	1.78 a	1.83 a	1.87 a	
	Average seedling height (cm) at 60 days			Mean
0	2.98 Aa	3.73 Aa	2.90 Aa	3.21 A
1	2.66 Aa	2.25 Ba	2.96 Aa	2.62 B
2	2.38 Aa	2.19 Ba	2.28 Aa	2.28 B
Mean	2.68 a	2.72 a	2.71 a	
	Cotyledonary Petiole (%) at 120 days			Mean
0	64.0 Aa	40.0 Aa	48.0 Aa	50.67 A
1	8.0 Ba	4.0 Ba	16.0 Ba	9.33 B
2	8.0 Ba	4.0 Ba	0.0 Ca	4.00 B
Mean	26.67 a	16.00 a	21.33 a	
	Root formation (%) at 120 days			Mean
0	0.0 Ba	0.0 Ba	0.0 Ba	0.00 B
1	8.0 Aa	8.0 Ba	4.0 Ba	6.66 A
2	16.0 Aa	28.0 Aa	16.0 Aa	20.00 A
Mean	8.00 a	12.00 a	6.67 a	
	Callus formation (%) at 120 days			Mean
0	0.0 Ca	0.0 Ba	0.0 Ba	0.00 C
1	16.0 Ba	44.0 Aa	0.0 Bb	20.00 B
2	60.0 Aa	28.0 Ab	28.0 Ab	38.67 A
Mean	25.33 a	24.00 a	9.33 b	

[z]Means followed by the same uppercase letter in each column and the same lowercase letter in each row do not differ by the Scott-Knott test at 5% probability.

In contrast to the other characteristics assessed, adding NAA to the culture medium provided increased average root formation, 6.66% and 20.0%, with 1 and 2 µM of NAA, respectively. Embryos cultured without NAA in culture medium did not form roots (Table 2).

The embryos grew in all the treatments, which was indicated by the elongated cotyledonary petioles that gave rise to shoot formation. Treatments using lower NAA concentrations had higher cotyledonary petiole growth and shoot formation but little root formation (Figure 4A, B and C). The amount of root formation increased as the NAA concentration increased, but cotyledonary petiole formation was reduced. (Figure 4D and H). Although 3 types of growth regulators were used in the culture medium, the percentage of callus formation was low but always present when using 2 µM of NAA (Figure 4I).

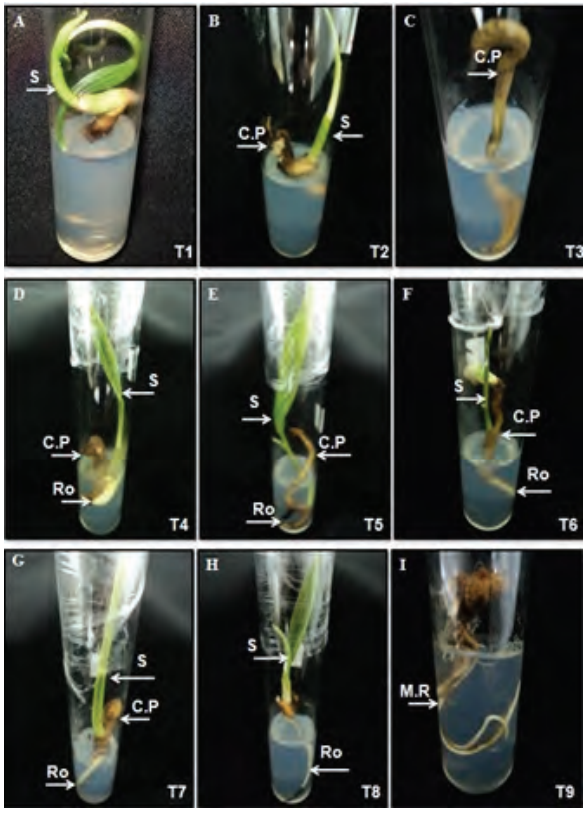

Figure 4. *In vitro* germination and growth of babassu (*Orbignya oleifera* Burret.) zygotic embryos cultured for 120 days in 50% MS medium, supplemented with 1 µM of BAP. The figure shows the various interactions between NAA and KIN; A) T1: BAP (1 µM), NAA (0 µM), and KIN (0 µM); B) T2: BAP (1 µM), NAA (0 µM), and KIN (1 µM); C) T3: BAP (1 µM), NAA (0 µM), and KIN (2 µM); D) T4: BAP (1 µM), NAA (1 µM), and KIN (0 µM); E) T5: BAP (1 µM), NAA (1 µM), and KIN (1 µM); F) T6: BAP (1 µM), NAA (1 µM), and KIN (2 µM); G) T7: BAP (1 µM), NAA (2 µM), and KIN (0 µM); H) T8: BAP (1 µM), ANA (2 µM), and KIN (1 µM), and I) T9: BAP (1 µM), ANA (2 µM), and KIN (2 µM). Shoot (S); cotyledon petiole (C.P); root (Ro); multiple roots (M.R).

Trial (III): Interaction between NAA and KIN in culture medium supplemented with BAP (2 μM) during in vitro germination and growth of babassu (Orbignya oleifera Burret.) zygotic embryos

The analysis of variance revealed that there were no significant effects on the following characteristics: germination, the germination speed index (GSI), shoot formation, and root formation. There were also no significant effects of the NAA x KIN interaction on the following characteristics: cotyledonary petiole formation, average seedling height, oxidation, and callus formation.

Using KIN in the culture medium favored an increased average seedling height during all the assessment periods (30, 60, 90, and 120 days of culture). The 1 and 2 μM KIN concentrations provided the highest average growth. A significant effect for using NAA in the culture medium was only observed at 30 days of culture, producing an average growth of 1.77 cm. Thus, addition of 2 μM BAP reduced the negative effect of NAA on the evaluated characteristics (Table 3).

Table 3. Average seedling height evaluated at 30, 60, 90, and 120 days of culture and cotyledonary petiole percentage, oxidation rate, and callus formation evaluated at 120 days of culture in babassu (*Orbignya oleifera* Burret.) zygotic embryos. The embryos were cultured *in vitro* with various concentrations of NAA and KIN, supplemented with 2 μM of BAP. Rio Verde, Goiás State, Brazil, 2011.

NAA (μM)	KIN (μM) 0	1	2	
	Average seedling height at 30 days (cm)			Mean
0	1.64 A[y]a[z]	1.77 Aa	1.89 Aa	1.77 A
1	1.32 Aa	1.62 Aa	1.47 Aa	1.47 B
2	1.00 Bb	1.58 Aa	1.54 Aa	1.37 B
Mean	1.31 b	1.64 a	1.63 a	
	Average seedling height at 60 days (cm)			Mean
0	2.11 Aa	2.36 Aa	2.21 Aa	2.22 A
1	1.91 Aa	2.38 Aa	2.23 Aa	2.17 A
2	1.50 Ab	2.42 Aa	2.08 Aa	2.01 A
Mean	1.83 b	2.39 a	2.18 a	
	Average seedling height at 90 days (cm)			Mean
0	2.25 Aa	2.45 Aa	2.39 Aa	2.36 A
1	1.91 Aa	2.53 Aa	2.40 Aa	2.27 A
2	1.53 Ab	2.67 Aa	2.17 Aa	2.14 A
Mean	1.88 b	2.56 a	2.33 a	
	Average seedling height at 120 days (cm)			Mean
0	2.35 Aa	2.50 Aa	2.53 Aa	2.46 A
1	1.97 Aa	2.82 Aa	2.63 Aa	2.47 A
2	1.59 Ab	3.36 Aa	2.67 Aa	2.56 A
Mean	1.96 b	2.95 a	2.61 a	
	Cotyledonary petiole (%) at 120 days			Mean
0	24.0 Aa	32.0 Aa	12.0 Aa	22.67 A
1	0.0 Ba	0.0 Ba	12.0 Ab	4.00 B
2	0.0 Ba	8.0 Ba	0.0 Aa	2.67 B
Mean	8.0 a	13.3 a	8.0 a	
	Oxidation (%) at 120 days			Mean
0	44.0 Aa	28.0 Aa	24.0 Aa	32.00 A
1	8.0 Ba	12.0 Ba	12.0 Aa	10.67 B
2	8.0 Bb	0.0 Bb	24.0 Aa	10.67 B
Mean	20.0 a	13.3 a	20.0 a	
	Callus formation (%) at 120 days			Mean
0	12.0 Ba	4.0 Ba	20.0 Aa	12.00 B
1	56.0 Aa	56.0 Aa	28.0 Ab	38.67 A
2	40.0 Aa	44.0 Aa	32.0 Aa	46.67 A
Mean	36.0 a	34.67 a	26.67 a	

[z]Means followed by the same uppercase letter in each column and the same lowercase letter in each row do not differ by the Scott-Knott test at 5% probability.

The rate of cotyledonary petiole formation was enhanced without NAA in culture medium, which also increased the oxidation rate. The percentage of cotyledonary petiole formation increased to 22.67%, whereas the oxidation rate increased to 32.0%. Thus, the addition of NAA prevents the oxidation of explants (Table 3).

In relation to callus formation, the highest concentrations provided higher average formation rates, 46.67 and 38.67%, in the culture medium containing 2 and 1 μM of NAA, respectively (Tabela 3).

Compared with the other trials (I and II), less shoot growth, more callus formation, and more oxidized explants were visually observed in all treatments of this trial (III). This result could be due to the higher growth regulator concentrations in the culture medium (Figure 5).

Cotyledonary petiole growth was stimulated without NAA in the culture medium (Figure 5A, B and C). However, increased NAA concentrations favored callus formation (Figure 5D and I). The calluses that formed were frail and white and/or cream colored, and some were oxidized (Figure 5F).

Based on the results from the comparison of the 3 trials, high NAA concentrations in the culture medium induced callus formation, where the callus formation increased with increasing BAP concentrations, as shown in trials (II) and (III). Cotyledonary petiole formation and average seedling height were favored without NAA in the culture medium, as shown in all 3 trials. Using 2 μM of BAP in the culture medium (trial III) oxidized the explants that did not form roots but formed calluses.

Therefore, the use of BAP at 1 μM (trial II) in the culture medium produced better results with respect to cotyledonary petiole formation and average seedling height. However, the use of 2 μM of NAA was favorable for root formation.

KIN did not have a significant effect on most of the characteristics that were assessed in the trials, but it had a significant effect on the cotyledonary petiole growth of the explants that formed calluses (trial III).

Therefore, we recommend the combination of 1 μM BAP and 2 μM NAA for the *in vitro* culture of zygotic embryos of babassu.

Hu and Ferreira (1998) reported that excised embryos that were at or close to maturity could germinate and grow in inorganic medium, in which growth regulators become dispensable. Ledo et al. (2007), studying dwarf coconut (*Cocos nucifera* L.) zygotic embryos, reported that NAA, BAP, and activated charcoal provided more root system and shoot growth. According to the studies by Asemota et al. (2007) of date palms (*Phoenix dactylifera* L.), the use of high NAA concentrations led to callus formation.

Based on the results obtained from these trials, it is apparent that the *in vitro* culturing of babassu (*Orbignya oleifera* Burret.) zygotic embryos is viable despite existing obstacles, such as removing the embryo from the endocarp without causing physical damage and the embryos' sensitivity to growth regulators.

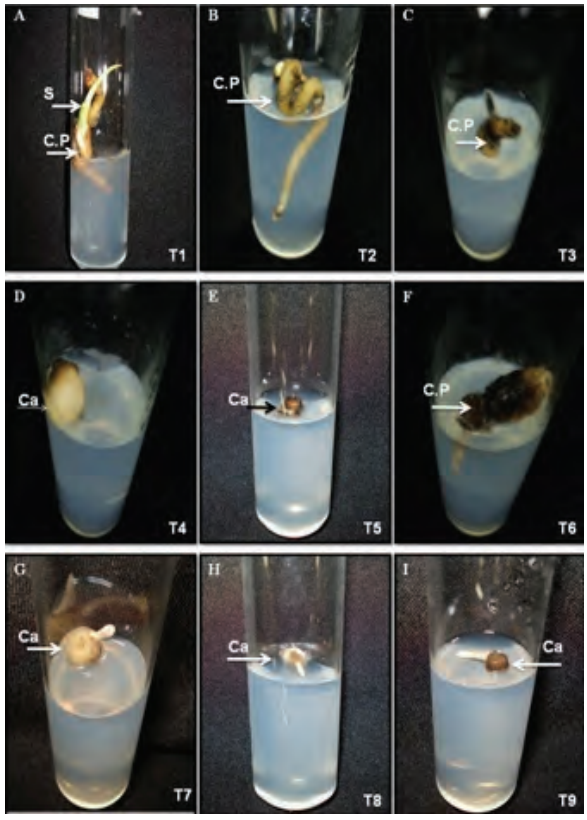

Figure 5. *In vitro* germination and growth of babassu (*Orbignya oleifera* Burret.) zygotic embryos cultured for 120 days in 50% MS medium, supplemented with 2 µM of BAP; A) T1: BAP (2 µM), NAA (0 µM), and KIN (0 µM); B) T2: BAP (2 µM), NAA (0 µM), and KIN (1 µM); C) T3: BAP (2 µM), NAA (0 µM), and KIN (2 µM); D) T4: BAP (2 µM), NAA (1 µM), and KIN (0 µM); E) T5: BAP (2 µM), NAA (1 µM), and KIN (1 µM); F) T6: BAP (2 µM), NAA (1 µM), and KIN (2 µM); G) T7: BAP (2 µM), NAA (2 µM), and KIN (0 µM); H) T8: BAP (2 µM), NAA (2 µM), and KIN (1 µM), and I) T9: BAP (2 µM), NAA (2 µM), and KIN (2 µM). Shoot (S); Cotyledonal petiole (C.P); Callus formation (Ca).

Conclusion

The best results for cotyledonary petiole growth and average seedling height were obtained using 1 μM of BAP in the culture medium.

A concentration of 2 μM NAA favored root formation.

The combination of 1 μM BAP and 2 μM NAA is recommended for the *in vitro* culture of zygotic embryos of babassu.

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