Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9275
ISSN on-line: 1807-8621
Doi: 10.4025/actasciagron.v35i2.15402

In vitro cultivation of zygotic embryos from Murici (*Byrsonima cydoniifolia* A. Juss.): establishment, disinfection, and germination

Cíntia de Oliveira Martendal[1], Murilo Martins Bernardino[2], Flávia Dionísio Pereira[3], Fabiano Guimarães Silva[2*], Carlos César Evangelista de Menezes[3] and Alessandra Cristina Boffino de Almeida Monteiro Hara[4]

*[1]Universidade Estadual de Maringá, Maringá, Paraná, Brazil. [2]Instituto Federal Goiano, Câmpus Rio Verde, Rod. Sul Goiana, Km 1, Cx. Postal 66, 75901-970, Rio Verde, Goiás, Brazil. [3]Cooperativa Agroindustrial dos Produtores Rurais do Sudoeste Goiano Ltda., Rio Verde, Goiás, Brazil. [4]Programa de Pós-Doutorado, Instituto Federal Goiano, Rio Verde, Goiás, Brazil. *Author for correspondence: E-mail: fabianoifgoiano@gmail.com*

ABSTRACT. The objective of this study is to establish an *in vitro* germination and cultivation protocol for murici (*Byrsonima cydoniifolia* A. Juss.) using zygotic embryos. Therefore, three assays were performed: in assay I, embryo asepsis was tested at exposure times of 5, 10, 15, 20, 25, and 30 minutes in 2.5% sodium hypochlorite, with or without immersion in 70% alcohol; in assay II, MS (MURASHIGE; SKOOG, 1962) e WPM (LLOYD; McCOWN, 1980) culture media were tested at salt concentrations of 25, 50, and 100%, with or without the addition of sucrose, to germinate the buds; in assay III, seedling growth was evaluated in MS and WPM culture media at salt concentrations of 25, 50 and 100%. Sodium hypochlorite (2.5%) with or without 70% alcohol was used to avoid contamination because it was not toxic to murici embryos. Water-agar was the most appropriate culture medium for bud germination, and 50% WPM was appropriate for seedling growth.

Keywords: tissue culture, asepsis, culture medium, Cerrado fruit.

Cultivo *in vitro* de embriões zigóticos de Murici (*Byrsonima cydoniifolia* A. Juss.): estabelecimento, desinfestação e germinação

RESUMO. Objetivou-se com este estudo, estabelecer um protocolo de germinação e cultivo *in vitro de* murici (*Byrsonima cydoniifolia* A. Juss.) a partir de embriões zigóticos. Para isso, foram feitos três ensaios: no ensaio I, testou-se a assepsia do embrião, nos tempos de exposição de 5, 10, 15, 20, 25 e 30 minutos no hipoclorito de sódio a 2,5% com e sem a imersão em álcool 70%. No ensaio II, para a germinação de embriões, testou-se os meios de cultivo MS (MURASHIGE; SKOOG, 1962) e WPM (LLOYD; McCOWN, 1980) nas concentrações de sais de 25, 50 e 100%, com e sem a adição de sacarose. No ensaio III, avaliou-se o crescimento de plântulas em meios de cultivo MS e WPM nas concentrações de sais de 25, 50 e 100%. Verificou-se que a imersão de 1 minuto em álcool 70% e 25 minutos em hipoclorito de sódio (2,5%) foi adequado para evitar contaminações não sendo fitotóxico para o embrião de murici. Para a germinação dos embriões, o meio de cultivo com água-ágar é o mais adequado e para o crescimento de plântulas é o meio de cultivo WPM 50%.

Palavras-chave: cultura de tecido, assepsia, meio de cultivo, frutífera do cerrado.

Introduction

Murici (*Byrsonima* ssp.) is a native fruit-producing plant of the Cerrado. It is a dicotyledon that belongs to the Malpighiaceae family. This tree species produces fruits between December and March in the highland regions of Southeastern Brazil, in the Cerrado of Mato Grosso and Goias, and on the coast of North and Northeastern Brazil. The mature fruits are yellow, 1.5 to 2 cm in diameter, have a marked odor, and are characterized as having an exotic flavor and aroma (ALVES; FRANCO, 2003; GUIMARÃES; SILVA, 2008; REZENDE; FRAGA, 2003).The fruits possess a high nutritional value and are mainly consumed fresh or in the form of juice, liquor, jelly, candy, or ice cream (ALMEIDA et al., 1998, 2008; FIGUEIREDO et al., 2005; GUIMARÃES; SILVA, 2008). The local populations that live off of extracting natural resources sell murici in open fairs and local markets (GUSMÃO et al., 2006); however, there is still no formal market.

Byrsonima cydoniifolia A. Juss is one of the species of murici. This small tree has the potential to produce food, wood, and popular medicine (GUSMÃO et al., 2006; SANNOMIYA et al., 2005); however, there is little information in the literature on murici species. Therefore, there is a need for more research on its

cultivation, which could aid its entry into the formal fruit market (CAVALCANTE et al., 2009).

The propagation of murici species is limited because the rate of germination is low, slow, and remarkably non-uniform (CARVALHO; NASCIMENTO, 2008; NOGUEIRA et al., 2004). This is because the embryos are covered by a thick endocarp that constitutes a mechanical barrier; in addition, another hypothesis proposes physiological dormancy (CARVALHO et al., 2007; SAUTU et al. 2006, 2007). Thus, the removal of the endocarp, extraction of the embryo, and the use of embryo cultivation techniques contributes to reduced germination time and allows for the production of plants that are free from contaminants. Although *in vitro* cultivation is a procedure that is employed for the grow of numerous species, knowledge of this technique in the native fruiting species in the Cerrado is still insufficient (LEDO et al., 2007; PINHAL et al., 2011).

An important alternative is the cultivation of *in vitro* embryos, which may be used as a tool for the cultivation of murici. This technique has been used to both overcome seed dormancy and to test viability, and it serves as a source of explants (HU; FERREIRA, 1998). In addition to serving as explants, buds resulting from the *in vitro* germination of zygotic embryos also form adventitious roots that allow for the subsequent transplanting of the seedlings to *ex vitro* conditions (SANTOS et al., 2005).

An important factor in the cultivation of embryos is the methodology of efficient asepsis, which decreases, as far as possible, the contaminants in the material (CORDER; BORGES JÚNIOR, 1999). The presence of microorganisms, particularly fungi and bacteria, may affect the germinative force and lead to poorly formed seedlings or seedling death. Therefore, it is important to use an appropriate and effective asepsis method so that the seedlings can serve as a source of explants that are free from contamination (COUTO et al., 2004).

The culture medium is another important factor for proper seedling growth. The culture media attempts to meet the needs of the studied species by providing the nutrients necessary for *in vitro* growth. Plant culture requirements vary according to species, cultivar, or explants, and should be experimentally determined for each particular case. Therefore, the appropriate amounts of nutrients and the osmotic relationship are factors that define germination and the subsequent stages of the growth process (KANASHIRO et al., 2009). According to Ledo et al. (2007) and Sousa et al. (2007), MS (Murashige and Skoog medium) (MURASHIGE; SKOOG, 1962) and WPM (woody plant medium) (LLOYD; McCOWN, 1980) are the culture media most widely used in studies on the *in vitro* germination of fruiting plants.

The controlled-environment germination of fruit-producing plants native to the Cerrado has been challenging due to various factors that interfere with the conservation and growth of these plants, such as contamination and the lack of protocols for *in vitro* cultivation. This process becomes even more difficult when large-scale changes are needed for growing plants at a commercial level (PINHAL et al., 2011).

Given the above and considering the lack of reports on the asexual propagation of *Byrsonima cydoniifolia* A. Juss., the objective of this study is to establish a germination and *in vitro* cultivation protocol for this species using zygotic embryos.

Material and methods

Collection of plant material

The plant material used for *in vitro* propagation was from *Byrsonima cydoniifolia* A. Juss. Ripe fruits were collected in November of 2010 in the municipality of Goiânia, Goiás State (S 16º 35.855' to W 049º 16.352'), at an altitude of 771 m (Figures 1A-C). The diaspores, which consist of the endocarp and the seed, were provided by the EMATER Training Center located on campus II of the Universidade Federal de Goiás. The diaspores were stored in paper bags at room temperature until they were needed in January 2011. All assays were performed in the Plant Tissue Culture Laboratory of the Federal Institute of Goiás (IFGoiano), Rio Verde Campus.

Figure 1. Plant material from *B. cydoniifolia*: (A) Bush. (B) Leaves and flowers. (C) Fruit. Rio Verde, Goiás State, 2011.
Photo: Cíntia de Oliveira Martendal.

Assay I: Influence of immersion time in a solution of sodium hypochlorite on the zygotic embryos of *B. cydoniifolia*

The selected diaspores were washed under running water for 5 minutes and then immersed in a solution of sodium hypochlorite containing 2.5% active chlorine (diluted from 100% commercial bleach) for 10 minutes. The diaspores were washed with autoclaved, distilled water. The excess water from the diaspores was removed with paper towels. The diaspores were

broken apart using a vice grip, and the embryos were carefully removed with tweezers to avoid any physical damage (Figures 2A-C).

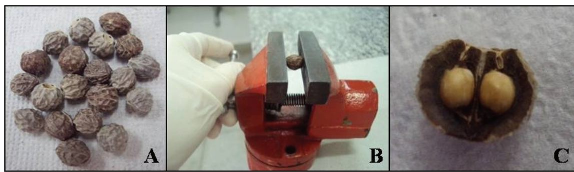

Figure 2. Plant material from *B. cydoniifolia*: (A) Diaspores. (B) The breaking of a diaspore in a vice grip. (C) A broken diaspore consisting of two embryos with integument. Rio Verde, Goiás State, 2011.
Photo: Cíntia de Oliveira Martendal.

To evaluate the effect of the disinfectant, the embryos remained submersed in a solution of 2.5% hypochlorite with the addition of three drops of Tween-20 detergent for 5, 10, 15, 20, 25, or 30 minutes, with or without immersion in 70% (v v^{-1}) alcohol for 30 seconds. Next, the embryos were washed three times in autoclaved, distilled water in a laminar flow cabinet; inoculated into test tubes (25 x 150 mm) containing 15 mL of a water-agar medium; and covered with plastic lids. To solidify the medium, 3.5 g L^{-1} of agar was used (Dinâmica®), and the pH was adjusted to 5.7 ± 0.3 before autoclaving at 120°C for 20 minutes. The test tubes containing the inoculated embryos were kept in a growth room at a temperature of 25°C ± 2ºC, a relative humidity of 45%, and a photoperiod of 16 hours. An active photosynthetic radiation of 45-55 μmol m^{-2} s^{-1} was obtained using fluorescent lamps.

The percentages of fungal and bacterial contamination were assessed daily, and on day 30, the percent germination, the height of the aerial part of the plants, and the root length were assessed. All embryos with radicle protrusion were considered germinated.

The experimental design was completely randomized in a 2 x 6 factorial arrangement (i.e., the presence and lack of alcohol x immersion times in sodium hypochlorite). Each treatment contained 20 replicates, with one test tube per repetition, for a total of 240 experimental units.

The results the contamination were expressed as percentages and underwent an arcsine transformation $(\%)^{0.5}$, and the rest , the length of the aerial part and length of the root, were calculated using $(x+1)^{0.5}$. The numerical data were statistically evaluated using analysis of variance. The means were tested using an F-test in a regression analysis at a 5% probability using SISVAR software (FERREIRA, 2011).

Assay II: Germination of the zygotic embryos of *B. cydoniifolia* grown in culture media with or without sucrose

The best conditions in assay (I) was used for embryo asepsis. The culture media used were MS (MURASHIGE; SKOOG, 1962) and WPM (LLOYD; McCOWN, 1980), at 25, 50 and 100% salt concentrations either with or without the addition of 30 g L^{-1} sucrose; water-agar was used for the control treatments. To solidify the medium, 3.5 g L^{-1} of agar was used (Dinâmica®), and the pH was adjusted to 5.7 ± 0.3 before autoclaving at 120°C for 20 minutes. The embryos were inoculated into test tubes (25 x 150 mm) containing 15 mL of culture media and kept in a growth room at a temperature of 25°C ± 2, a relative humidity of 45%, and a photoperiod of 16 hours. An active photosynthetic radiation of 45-55 μmol m^{-2} s^{-1} was obtained using fluorescent lamps.

The percent germination and the germination velocity index (GVI) were assessed daily, and on day 30, the height of the aerial part was also assessed. GVI was calculated according to the method used by Maguirre (1962). All embryos exhibiting radicle protrusion were considered germinated.

The experimental design was completely random, with seven treatments and 20 repetitions each. One test tube was used for each repetition for a total of 140 experimental units. The results, the contamination and germination, expressed in percentages, underwent an arcsine transformation $(\%)^{0.5}$, and the rest the length of the aerial part and GSI, were calculated using $(x+1)^{0.5}$. The numerical data were statistically evaluated using analysis of variance, and the means were tested by a Scott-Knott test at a 5% probability using SISVAR software (FERREIRA, 2011).

Assay III: Cultivating the seedlings of *B. cydoniifolia* in different culture media

Seedlings grown for 30 days were used as a source of explants. These were derived from the zygotic embryos that had been pre-established *in vitro*. The cotyledons and the roots of the seedlings were excised, resulting in explants of ± 1 cm in length. The explants were inoculated into bottles containing 50 mL of MS or WPM media at salt concentrations of 25, 50, or 100%, plus 30 g L^{-1} of sucrose. To solidify the medium, 3.5 g L^{-1} of agar were used, and the pH was adjusted to 5.7 ± 0.3 before autoclaving at 120°C for 20 minutes. The bottles containing the inoculated explants were kept in a growth room at a temperature of 25°C ± 2, a relative humidity of 45%, and a photoperiod of 16 hours. An active photosynthetic radiation of 45-55 μmol m^{-2} s^{-1} was obtained using fluorescent lamps.

At 30 and 60 days of growth, the contamination percentage, the length of the aerial part, and the number of leaves were evaluated. At 60 days, the length of the aerial part and the number of leaves per seedling were measured.

The experimental design was completely randomized in a 2 x 3 factorial arrangement (culture media x salt concentrations), and each treatment contained 20 repetitions consisting of one test tube per assay for a total of 120 experimental units. The numerical data were statistically evaluated using analysis of variance, and the means were tested by a Scott-Knott test at a 5% probability using SISVAR software (FERREIRA, 2011).

Results and discussion

Murici embryos are a whitish-yellow color, and their greatest peculiarity is their spiral shape (Figure 3A). If the spiral shape changes upon inoculation, the germination rate tends to fall, which compromises regeneration. Therefore, it is of paramount importance that the tegument is wrapped around the embryo during the disinfection stage because the tegument helps to maintain the shape and serves to protect the embryo from external exposure. During the disinfection process, the tegument tends to become damaged and may be completely broken, exposing the embryos. Therefore, the embryos selected for inoculation were those that remained intact, thus maintaining their spiral shape.

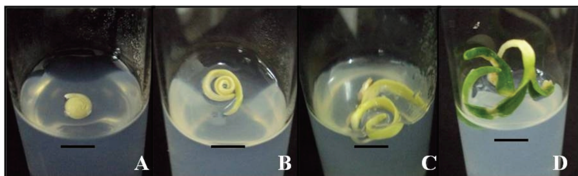

Figure 3. *In vitro* germination and growth of a zygotic embryo from *B. cydoniifolia*: (A) Embryo on day 1 of cultivation. (B) Embryo with root protrusion after day 7 of cultivation. (C) Seedling formation at day 14 of cultivation. (D) Seedling at *in vitro* cultivation day 30. Rio Verde, Goiás State, 2011. Bar: 11 mm.
Photo: Cíntia de Oliveira Martendal.

The injected embryos remained spiraled up until the moment that the soaking process was complete, at which point the embryos unraveled by themselves and began the protrusion of their roots, which began on day 7 of cultivation (Figure 3B). After a few days, the cotyledons of the embryos emerged and gradually changed color. At this time, chlorophyll was produced, and the embryos began turning green. The greening process became more evident beginning on day 14 of *in vitro* cultivation (Figure 3C). On day 30 of cultivation, well-formed seedlings were obtained that had an intense green color, which is characteristic of this species (Figure 3D). During the entire germination process there was no oxidation of the explants and no formation of calluses observed. An average of 85% of the embryos germinated for all treatments (data not shown).

Assay I: Influence of immersion time in a solution of sodium hypochlorite on the zygotic embryos of *B. cydoniifolia*

A quadratic regression model displayed the best fit in response to the assays tested. Moreover, when disinfection was performed with 70% alcohol followed by bleach for 25 minutes, the contamination percentage was 5%. When the embryos were not immersed in 70% alcohol, the optimal asepsis time using bleach was 30 minutes, in which 5% contamination was also obtained (Figure 4A).

It was determined that embryos submerged in bleach for only 5 minutes displayed the highest percentage of contamination (i.e., 20%), regardless of whether the embryos were immersed in 70% alcohol (Figure 4A).

Thus, lower contamination rates were generally observed with a longer exposure of embryos to bleach (2.5% sodium hypochlorite) with or without previous immersion in alcohol. Maximal contamination was observed with immersion times of 7.99 minutes in bleach without alcohol (18.83% contamination) and 7.41 minutes in bleach with alcohol (37.55% contamination).

With regard to the average height of the aerial part, disinfection with bleach alone for five minutes produced the best results, with heights averaging 1.6 cm. When embryos were subjected to the combination of 70% alcohol and bleach for five minutes, the aerial part grew approximately 1.2 cm (Figure 4B). The height of the aerial part decreased with longer periods of immersion in bleach without alcohol up to 20 minutes; beyond 20 minutes, the height remained the same. The duration of immersion in alcohol did not change the height of the aerial part. The average height of the aerial part reached a maximum of 1.02 cm with 31.25 minutes of immersion in bleach without alcohol and a maximum of 1.16 cm in bleach with alcohol after 8.67 minutes of immersion.

The average root length decreased with increasing immersion time up to 20 minutes, but the root lengths actually increased with times of 25 and 30 minutes (Figure 4C). Bleach with 70%

alcohol produced a maximum root length of 0.9 cm with a 25-minute immersion. When asepsis was performed only with bleach, the greatest root length was 0.7 cm with a 15-minute immersion. Maximal average root lengths were observed with 18 minutes of immersion in bleach without alcohol (0.41 cm) and with 16.18 minutes of immersion in bleach without alcohol (0.39 cm).

Taking into consideration all of the above features, a one-minute immersion in 70% alcohol followed by a 25-minute immersion in bleach (2.5% sodium hypochlorite) is the optimal method for efficient, nontoxic disinfection of murici embryos.

Different methodologies for the asepsis of murici embryos have been used previously. For example, a study on small-murici (*Byrsonima intermedia* A. Juss.) by Nogueira et al. (2004) used 70% alcohol for 30 seconds and then a solution of 0.5% sodium hypochlorite for 5 minutes for embryos with the tegument intact. After the tegument was removed, the embryos were again immersed in a 0.5% sodium hypochlorite solution for 5 minutes.

Castro et al. (2005), performed asepsis on only the diaspores of murici (*Byrsonima verbascifolia* Rich.) by disinfecting with commercial 2% sodium hypochlorite for 10 minutes, followed by the extraction of the embryos from the endocarp and their inoculation into test tubes in a laminar flow cabinet.

Sodium hypochlorite and alcohol are two disinfecting agents that are widely used for the asepsis of seeds and embryos. Both have satisfactory effects against fungal and bacterial contamination.

Assay II: Germination of the zygotic embryos from *B. cydoniifolia* A. Juss. in culture media with or without sucrose

Asepsis performed in bleach (2.5% sodium hypochlorite) was efficient, maintained the integrity of the embryos, and had no toxic effects. Consequently, embryos germinated in all treatments (Table 1).

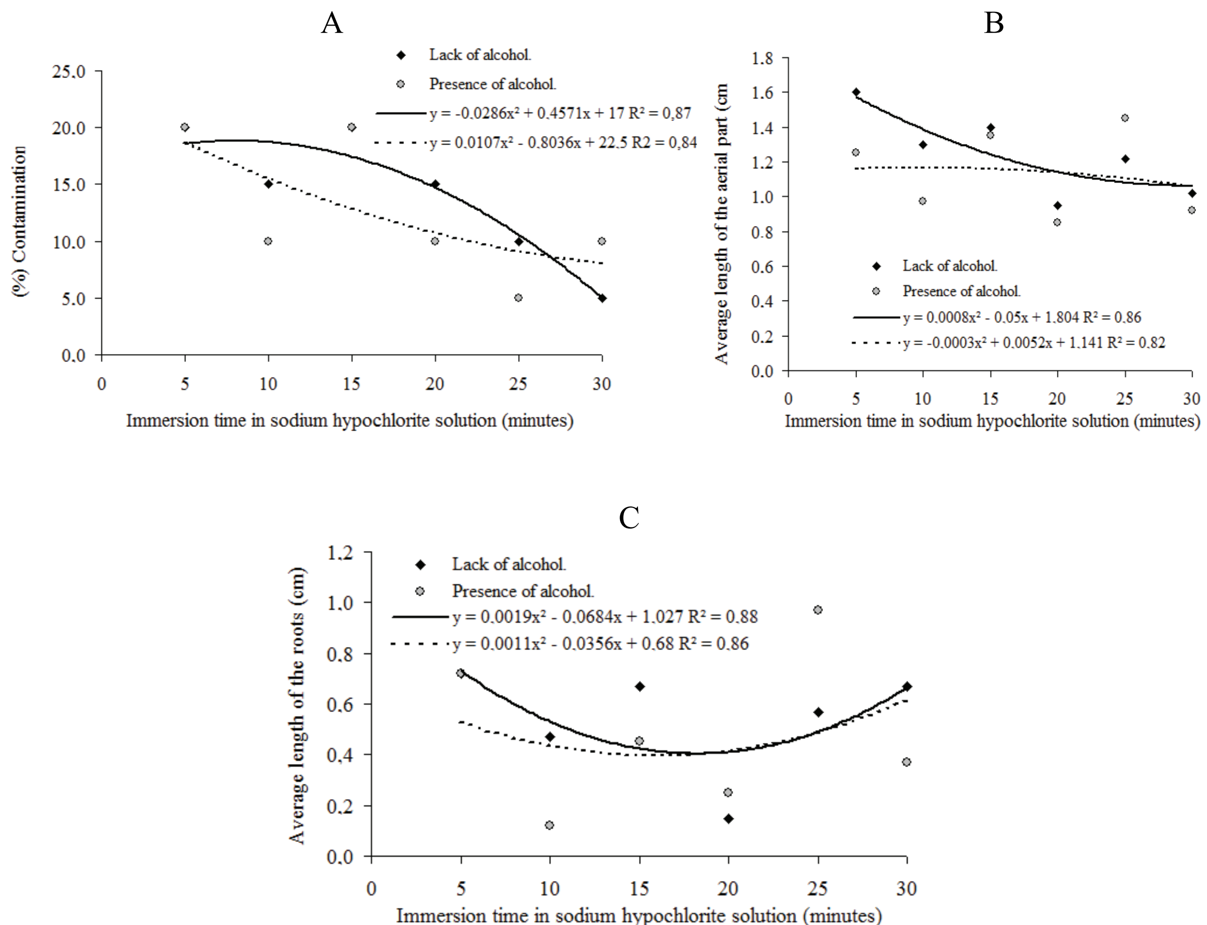

Figure 4. Asepsis of the zygotic embryos of *B. cydoniifolia* at day 30 of *in vitro* cultivation: (A) Contamination percentage as a function of the different immersion times in a 2.5% solution of sodium hypochlorite. (B) The average length of the aerial part as a function of the concentration of sodium hypochlorite. (C) The average length of the roots as a function of the concentration of sodium hypochlorite. Rio Verde, Goiás State, 2011.

Table 1. Germination, germination speed index (GSI), and the average length of the aerial part of *B. cydoniifolia* A. Juss. as a function of the salt concentration in the MS and WPM media without sucrose and the water-agar control treatment after 30 days of cultivation. Rio Verde, Goiás State, 2011.

Culture medium	Germination (%)	GSI (%)	Length of the aerial part (cm)
Water-Agar	95 a	7 a	2.17 a
25% MS with sucrose	85 a	8 a	1.58 a
50% MS with sucrose	45 b	8 a	0.80 b
100% MS with sucrose	50 b	6 a	1.32 b
25% WPM with sucrose	75 a	7 a	1.81 a
50% WPM with sucrose	75 a	8 a	2.05 a
100% WPM with sucrose	65 a	8 a	1.73 a

[Z]The means followed by the same letter in the same column are significantly different according to a Scott-Knott test at a 5% probability

Sucrose addition to the various concentrations of culture media was important for increased germination percentage and the length of the aerial part (Table 1). None of the investigated characteristics changed when the concentration and type of culture media were tested without the addition of sucrose.

In general, the greatest germination rates occurred as a function of reduced salt concentrations in the culture media. The overall germination rate varied between 45 and 95%. The lowest germination rates were 45% and 50% which corresponded to the 50 and 100% MS media, respectively (Table 1).

The length of the aerial part was similar to the tendency observed for the germination percentage. The shortest aerial part lengths were observed in the 50 and 100% MS treatments, with an average length of 0.80 cm and 1.32 cm, respectively. The other treatments the length of the aerial part obtained averages of 1.58 cm to 2.17 cm (Table 1). There was no difference in the root length among the tested media. The average root length in the media without sucrose was 1.33 cm, and the average root length in the media with sucrose was 1.15 cm (data not shown).

Furthermore, we observed that the media prepared only with water and agar performed well in terms of all characteristics evaluated, revealing the possibility of germination in this medium without the need to include salts or sucrose. This possibility would be beneficial because it reduces production cost.

Physiologically, there is no harm to the embryos because the germination process occurs as a function of the nutritional reserves in the cotyledons, which are present in the excised embryos and may or may not use the nutritional sources present in the culture medium. According to Hu and Ferreira (1998), embryos excised at a mature or nearly mature state are almost autotrophic, and in general, these embryos eliminate the supplemental energy source. The results of the Hu and Ferreira (1998) study were confirmed by Ribeiro et al. (1998). This group tested different concentrations of sucrose on sweet orange (*Citrus sinensis* Osb.) embryos and observed high survival rates among the embryos at all tested levels, showing that sucrose is expendable during the initial stage of embryo development in this species. Low concentrations of sucrose are also indicated for the germination of zygotic embryos from murmuru (*Astrocaryum ulei*); for these embryos, concentrations below 15 g L^{-1} of sucrose are most appropriate (PEREIRA et al., 2006).

In contrast, Castro et al. (2005) suggested the use of 100% MS supplemented with 30 g L^{-1} of sucrose for the *in vitro* cultivation of zygotic embryos from *Byrsonima verbascifolia* Rich. Nogueira et al. (2004) proposed the use of 50% MS or WPM media without sucrose for the *in vitro* cultivation of zygotic embryos from *Byrsonima intermedia* A. Juss.

Assay III: Cultivation of B. cydoniifolia seedlings in different culture media

In preliminary assays (data not shown), we observed that due to the shape of the embryos, the seedlings bend and lose their direct contact with the culture medium. Therefore, it is necessary to excise part of the cotyledon leaves and root to keep the explants straight, which facilitates inoculation (Figures 5A-C). The excision of the cotyledon leaves and root allows the primary leaves to emerge sooner. Additionally, it is important that the central axis of the explants are inoculated above the culture medium. Overlapping the central axis does not allow new leaf growth and consequently leads to the loss of plant material (Figure 5D).

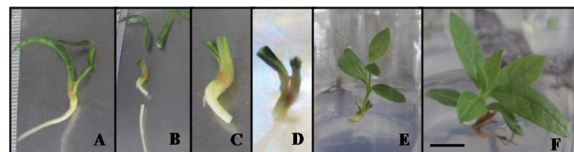

Figure 5. *B. cydoniifolia*: (A) Seedling at day 30 of *in vitro* cultivation. (B) Seedling with the excision of the cotyledon leaves and root. (D) Excised seedling in the culture medium. (E) Seedling after 30 days of cultivation. (F) Seedling after 60 days of cultivation. Rio Verde, Goiás State, 2011. Bar: 10 mm.
Photo: Cíntia de Oliveira Martendal.

Across all treatments, after 30 days of *in vitro* cultivation, we observed well-formed seedlings with intense green coloration, which is characteristic of the species. There was no oxidation and no callus formation (Figure 5E). We also observed that both the MS and WPM medium induced an increase in the length of the aerial part; however, for the tested

salt concentrations, the WPM medium provided the best results at concentrations of 25% and 50%, with average aerial part lengths of 2.95 cm and 3.50 cm, respectively (Table 2).

Table 2. Length of the aerial part (cm), the root (cm), and the number of leaves per seedling of *B. cydoniifolia* at day 30 of cultivation in relation to the concentration of salts in the MS and WPM media. Rio Verde, Goiás State, 2011.

Concentration	Culture medium MS	WPM
	Length of the aerial part (cm) after 30 days of cultivation	
25%	1.81 Ab[Z]	2.95 Aa
50%	2.09 Ab	3.50 Aa
100%	2.27 Aa	2.50 Aa
	Number of leaves per seedling after 30 days of cultivation	
25%	3.25 Ab	5.30 Aa
50%	4.62 Aa	5.90 Aa
100%	4.27 Aa	4.57 Aa

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

There was no significant difference between the different media used and the number of leaves per seedling (Table 2). The 25% MS medium produced the lowest number of leaves, which was an average of 3.25. The salt concentrations tested in the WPM medium yielded the best responses. An average of 4.57, 5.30, and 5.90 leaves were obtained for the 100, 25, and 50% salt concentrations, respectively.

After 60 days, there was up to 131.42% increased growth of the aerial part compared with 30 days of *in vitro* cultivation. Upon evaluating the different types of culture medium used, no significance was identified (Table 3). In the WPM medium, the aerial part lengths observed at the different salt concentrations were not significantly different from one another; however, they did provide the best averages: 3.78 cm, 4.40 cm, and 4.60 cm for the 100, 25, and 50% concentrations, respectively.

Table 3. Length of the aerial part (cm) and the number of leaves per seedlings of *B. cydoniifolia* after 60 days of cultivation in relation to the concentration of salts in the MS and WPM media. Rio Verde, Goiás State, 2011.

Concentration	Culture medium MS	WPM
	Length of the aerial part (cm) after 60 days of cultivation	
25%	3.00 Ab[Z]	4.40 Aa
50%	2.93 Ab	4.60 Aa
100%	2.75 Aa	3.78 Aa
	Number of leaves per seedling after 60 days of cultivation	
25%	8.28 Aa	10.00 Aa
50%	7.28 Aa	8.90 Aa
100%	7.50 Aa	5.71 Ba

[Z]The means followed by the same uppercase letter in each column, and by the same lowercase letter in each row, do not significantly differ according to a Scott-Knott test at a 5% probability

There was a significant difference between the different types of culture medium and the number of leaves per seedling. The 100% WPM medium produced the lowest number of leaves per seedling, with an average of 5.71 (Table 3).

In general, the WPM medium at low salt concentrations provided the best results for all evaluated characteristics; therefore, we advocate the use of 50% WPM.

According to Grattapaglia and Machado (1998), culture media with more diluted macronutrients provide the best results for woody species. Similarly, Mello et al. (1999) found that WPM culture medium was superior to MS and DKW (Driver-Kuniyuki walnut) media for the *in vitro* establishment of acerola (*Malpighia emarginata* DC.). This group observed that the best results were obtained by explants inoculated into the WPM medium. In the work of Silva et al. (2006), WPM culture medium also provided the best results for the *in vitro* establishment of the rabbiteye blueberry plant (*Vaccinium ashei* Reade).

Based on the results of our experiments, we determined that the *in vitro* cultivation of murici (*Byrsonima cydoniifolia* A. Juss.) embryos is possible, despite some obstacles, such as contamination and the difficulty of removing the embryo from the diaspore without causing physical damage. In general, the culture media with the lowest salt concentrations provided the best results for the evaluated characteristics.

Conclusion

The asepsis of *B. cydoniifolia* embryos should be performed using 70% alcohol for 1 minute followed by the application of sodium hypochlorite (2.5%) for 25 minutes. For the germination of *B. cydoniifolia* embryos, a water and agar culture medium is the most appropriate, and the embryos should remain in this medium for 30 days. For the growth of *B. cydoniifolia* seedlings, the most appropriate culture medium is 50% WPM.

Acknowledgements

The authors would like to thank the Research Support Foundation of the Goias State (FAPEG), CAPES, and PIBIC programs for their financial support. The authors also thank the EMATER Training Center for providing the plant material.

References

ALMEIDA, S. P.; COSTA, T. S. A.; SILVA, J. A. Frutas nativas do Cerrado: caracterização físico-química e fonte

potencial de nutrientes. In: SANO, S. M.; ALMEIDA, S. P.; RIBEIRO, J. F. (Ed.). **Cerrado**: ecologia e flora. Brasília: Embrapa Informação Tecnológica, 2008. p. 351-381.

ALMEIDA, S. P.; PROENÇA, C. E. B.; SANO, S. M.; RIBEIRO, J. F. **Cerrado**: espécies vegetais úteis. Planaltina: Embrapa-CPAC, 1998.

ALVES, G. L.; FRANCO, M. R. B. Headspace gas chromatography–mass spectrometry of volatile compounds in murici (*Byrsonima crassifolia* L. Rich). **Journal of Chromatography**, v. 985, n. 4, p. 297-301, 2003.

CARVALHO, J. E. U.; NASCIMENTO, W. M. O. Caracterização dos pirênios e métodos para acelerar a germinação de sementes de murici do clone Açu. **Revista Brasileira de Fruticultura**, v. 30, n. 3, p. 775-781, 2008.

CARVALHO, J. E. U.; NASCIMENTO, W. M. O.; MULLER, C. H. Propagação do Murucizeiro (*Byrsonima crassifolia* (L.) Rich.). In: CARVALHO, J. E. U.; NASCIMENTO, W. M. O.; MULLER, C. H. (Ed.). **Produção de mudas de espécies frutíferas nativas da Amazônia**. Fortaleza: Instituto Frutal, 2007. p. 87-99.

CASTRO, A. H. F.; ALVARENTA, A. A.; PAIVA R.; GOMES, G. A. C. Propagação do murici (*Byrsonima verbascifolia*) por cultivo *in vitro* de embriões. **Plant Cell Culture and Micropropagation**, v. 1, n. 1, p. 1-7, 2005.

CAVALCANTE, T. R. M.; NAVES, R. V.; FRANCESCHINELLI, E. V.; SILVA, R. P. Polinização e formação de frutos em araticum. **Bragantia**, v. 68, n. 1, p. 13-21, 2009.

CORDER, M. P. M.; BORGES JÚNIOR, N. Desinfestação e quebra de dormência de sementes de *Acacia mearnsii* de Wild. **Ciência Florestal**, v. 9, n. 2, p. 1-7, 1999.

COUTO, J. M. F.; OTONI, W. C.; PINHEIRO, A. L.; FONSECA, E. P. Desinfestação e germinação in vitro de sementes de Mogno (*Swietenia macrophylla* King). **Revista Árvore**, v. 28, n. 5, p. 633-642, 2004.

FERREIRA, D. F. Sisvar: um sistema computacional de análise estatística. **Ciência e Agrotecnologia**, v. 35, n. 6, p. 1039-1042, 2011.

FIGUEIREDO, M. E.; MICHELIN, D. C.; SANNOMIYA, M.; SILVA, M. A.; SANTOS, L. C.; ALMEIDA, L. F. R.; SOUZA BRITO, A. R. M.; SALGADO, H. R. N.; VILEGAS, W. Avaliação química e da atividade antidiarréica das folhas de *Byrsonima cinera* DC. (Malpighiaceae). **Revista Brasileira de Ciências Farmacêuticas**, v. 41, n. 1, p. 79-83, 2005.

GRATTAPAGLIA, D.; MACHADO, M. A. Micropropagação. In: TORRES, A. C.; CALDAS, L. S.; BUSO, J. A. (Ed.). **Cultura de tecidos e transformação genética de plantas**. Brasília: Embrapa-SPI; Embrapa-CNPH, 1998. v. 1, p. 183-260.

GUIMARÃES, M. M.; SILVA, M. S. Valor nutricional e características químicas e físicas dos frutos de murici-passa (*Byrsonima verbascifolia*). **Ciência e Tecnologia de Alimentos**, v. 28, n. 4, p. 817-821, 2008.

GUSMÃO, E. F.; VIEIRA, A.; FONSECA JÚNIOR, E. M. Biometria de frutos e endocarpos de murici (*Byrsonima verbascifolia* Rich. ex A. Juss.). **Cerne**, v. 12, n. 1, p. 84-91, 2006.

KANASHIRO, S.; RIBEIRO, R. C. S.; GONÇALVES, A. N.; DEMÉTRIO, V. A.; JOCYS, T.; TAVARES, A. R. Effect of calcium on the *in vitro* growth of *Aechmea blanchetiana* (Baker) L.B. Smith plantlets. **Journal of Plant Nutrition**, v. 32, n. 1, p. 867-877, 2009.

HU, C. Y.; FERREIRA, A. G. *In vitro* embriology of *Ilex*. In: TORRES, A. C.; CALDAS, L. S.; BUSO, J. A. (Ed.). **Cultura de tecidos e transformação genética de plantas**. Brasília: Embrapa SPI; Embrapa CNPH, 1998. v. 2. p. 371-393.

LEDO, A. S.; SECA, G. S. V.; BARBOZA, S. B. S. C.; SILVA JUNIOR, J. F. Crescimento inicial de mangabeira (*Hancornia speciosa* Gomes) em diferentes meios de cultivo *in vitro*. **Ciência e Agrotecnologia**, v. 31, n. 4, p. 989-993, 2007.

LLOYD, G.; McCOWN, B. Commercially-feasible micropropagation of Mountain laurel, *Kalmia latifolia*, by use of shoot tip culture. **International Plant Propagation Society Proceedings**, v. 30, n. 1, p. 421-427, 1980.

MAGUIRRE, J. D. Speed of germination AID in selection and evaluation for seedling and vigour. **Crop Science**, v. 2, n. 2, p. 176-177, 1962.

MELLO, N. F.; OKASAKI, W. Y.; LEITE, C. B.; FÁRI, M. Estabelecimento do cultivo *in vitro* da aceroleira (*Malpighia emarginata* DC.). **Ciência e Agrotecnologia**, v. 23, n. 1, p. 102-107, 1999.

MURASHIGE T.; SKOOG, F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. **Physiologia Plantarum**, v. 15, n. 3, p. 473-497, 1962.

NOGUEIRA, R. C.; PAIVA, R.; CASTRO, A. H.; VIEIRA, C. V.; ABBADE, L. C.; ALVARENGA, A. A. Germinação *in vitro* de murici-pequeno (*Byrsonima intermédia* A. Juss.). **Ciência e Agrotecnologia**, v. 28, n. 5, p. 1053-1059, 2004.

PEREIRA, J. E. S.; MACIEL, T. M. S.; COSTA, F. H. S.; PEREIRA, M. A. A. Germinação *in vitro* de embriões zigóticos de murmuru (*Astrocaryum ulei*). **Ciência e Agrotecnologia**, v. 30, n. 2, p. 251-256, 2006.

PINHAL, H. F.; ANASTÁCIO, M. R.; CARNEIRO, P. A. P.; SILVA, V. J.; MORAIS, T. P.; LUZ, J. M. Q. Aplicações da cultura de tecidos vegetais em fruteiras do Cerrado. **Ciência Rural**, v. 41, n. 7, p.1136-1142, 2011.

REZENDE, C. M.; FRAGA, S. R. Chemical and aroma determination of the pulp and seeds of murici (*Byrsonima crassifolia* L.). **Journal of the Brazilian Chemical Society**, v. 14, n. 3, p. 425-428, 2003.

RIBEIRO, V. G.; PASQUAL, M.; RAMOS, J. D.; VICHIATO, M.; SANÁBIO, D. Cultivo *in vitro* de embriões de Laranja 'Pera': concentrações do meio MS e sacarose. **Ciência e Agrotecnologia**, v. 22, n. 4, p. 429-434, 1998.

SANNOMIYA, M.; MICHELIN, D. C.; RODRIGUES, E. M.; SANTOS, L. C.; SALGADO, H. R. N. *Byrsonima crassa* Niedenzu (IK): antimicrobial activity and chemical study. **Revista de Ciências Farmacêuticas Básica e Aplicada**, v. 26, n. 1, p. 71-75, 2005.

SANTOS, C. G.; PAIVA, R.; PAIVA, P. D. O.; SANTANA, J. R. F.; PEREIRA, A. B. Propagação de *coffea*

arabica c.v. acaiá Cerrado por meio do cultivo *in vitro* de embriões. **Plant Cell Culture and Micropropagation**, v. 1, n. 1, p. 19-23, 2005.

SAUTU, A.; BASKIN, J. M.; BASKIN, C. C.; CONDIT, R. Studies on the biology of 100 native species of trees in a seasonal moist tropical forest, Panama, Central Americ. Maryland. **Forest Ecology Management**, v. 234, n. 1, p. 245-263, 2006.

SAUTU, A.; BASKIN, J. M.; BASKIN, C. C.; DEAGO, J.; CONDIT, R. Classification and ecological relationships of seed dormancy in a seasonal moist tropical forest, Panama, Central America. **Seed Science Research**, v. 17, n. 2, p. 127-140, 2007.

SILVA, L. C. S.; SCHUCH, M. W.; SOUZA, J. A.; ERIG, A. C.; ANTUNES, L. E. C. Meio nutritivo, reguladores de crescimento e frio no estabelecimento *in vitro* de mirtilo (*Vaccinium ashei* Reade) cv. Delite. **Revista Brasileira de Agrociência**, v. 12, n. 4, p. 405-408, 2006.

SOUSA, C. S.; MOREIRA, A. J. S.; BASTOS, L. P.; COSTA, M. A. P. C.; ROCHA, M. A. C.; HANSEN, D S. Germinação e indução de brotações *in vitro* utilizando diferentes reguladores vegetais em mangabeira (*Hancornia speciosa*). **Revista Brasileira de Biociências**, v. 5, n. 2, p. 276-278, 2007.

Received on November 30, 2011.
Accepted on March 31, 2012.

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.