academicJournals

Vol. 15(33), pp. 1771-1780, 17 August, 2016
DOI: 10.5897/AJB2016.15417
Article Number: 35E05EC60118
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Micropropagation of caçari under different nutritive culture media, antioxidants, and levels of agar and pH

Maria Da Conceicao Da Rocha Araujo[1]*, Edvan Alves Chagas[2], Maria Isabel Ribeiro Garcia[3], Sara Thiele Sobral Pinto[3], Pollyana Cardoso Chagas[3], Wagner Vendrame[4], Adamor Barbosa Mota Filho[3] and Olisson Mesquita de Souza[3]

[1]Post-graduation program in Biodiversity and Biotechnology - Rede Bionorte/UFAM/UFRR, Roraima, Brazil.
[2]Brazilian Agricultural Research Corporation (Embrapa), Brazil. Productivity Research Scholarship – CNPq.
[3]Agricultural Science Center, Federal University of Roraima, Roraima, Brazil.
[4]Tropical Research and Education Center, Homestead (TREC-UF), University of Florida, Florida-EUA.

Received 18 April, 2016; Accepted 8 August, 2016

The caçari (*Myrciaria dubia*) is a native fruit tree from Amazon with high concentrations of vitamin C. This study aimed to adjust a culture medium that meets the nutritional needs for the *in vitro* development of caçari, evaluating the effect of different concentrations and nutritive culture media, antioxidant, and levels of agar and pH. Three experiments were carried out in a completely randomized design: 1 - woody plant medium (WPM), Murashige and Skoog (MS) and Juan, Antonio, Diva and Silvia medium (JADS) nutritive media with 25, 50, 75 and 100% concentrations; 2 - pH (3.7, 4.7, 5.7 and 6.7) and agar concentrations (0, 3.5, 7.0 and 10.5 g.L^{-1}); 3 - Antioxidants [(ascorbic acid (AA), citric acid (CA), polyvinylpyrrolidone (PVP)] and concentrations (0, 100, 200, 300 mg.L^{-1}) on the control of phenolic oxidation of stem segments. After collection, the explants were disinfested in a laminar flow chamber, dipping in 70% ethanol for 3 min and 1.5% sodium hypochlorite for 12 min, followed by three washes in distilled and autoclaved water. After disinfestation, the explants were inoculated in 15 × 125 mm test tubes containing 30 mL culture medium, according to each experiment and their respective treatments. After 90 days, the number and length of sprouts (cm) and the oxidation were evaluated. The best results were obtained using the WPM medium at a concentration of 100% with 7 g.L^{-1} agar, and pH adjusted to 5.7. The use of antioxidants in the tested conditions did not contribute to decrease in oxidation in explants, indicating that there is no need of adding them into the culture medium.

Key words: Camu-camu, *in vitro* culture, *Myrciaria dubia,* organogenesis.

INTRODUCTION

The caçari (*Myrciaria dubia* (Kunth.) McVaugh) is a native fruit tree from Amazon, widely distributed in the conditions of the Northern Amazon. This species has aroused a great interest of the national and international

*Corresponding author. E-mail: nilmacoly@hotmail.com

market due to its high content of vitamin C, which may reach 7,355.20 mg per 100^{-1} g of ascorbic acid pulp (Chagas et al., 2015), and nutraceutical compounds important for our health (Neves et al., 2015).

Because the species is still in domestication process (Chagas et al., 2012), there is little information about its micropropagation. This technique is important for use in breeding programs of the species, multiplication of high clones with difficult rooting and obtaining plants free from pests and diseases and with high quality. Thus, one of the most important factors to be studied, that most influences and determines the success of the *in vitro* culture, is the nutritional balance of the culture medium and its interaction with the genetic material or explant. In most cases the culture medium is empirically defined, using as a basis the protocoled culture media, showing no specificity to the species nutritional requirements, the limiting factor of the morphogenetic processes of a given species (Correia, 2006).

Many culture media formulations have been made in the last 100 years, such as the Murashige and Skoog (MS) medium (Murashige and Skoog, 1962), to optimize the culture of roots, cells, calli, among others, representing one of the most widespread and used media for herbaceous species. This medium has a high total ionic concentration, with a high concentration of nitrogen, potassium, zinc and chlorine; in comparison to other media (Leifert et al., 1995). For woody species, media with more diluted mineral balance have been more used, such as the WPM culture medium (Lloyd and Mccown, 1981) which has lower salt concentrations, in particular, the nitrogen and potassium (Rocha, 2005). The Juan, Antonio, Diva and Silvia (JADS) medium (Correia et al., 1995), similar to the Woody plant medium (WPM), also has low nitrogen, potassium, and molybdenum content.

The culture medium consistency is another important factor for the *in vitro* culture and plays a fundamental effect on the morphogenesis and growth of sprouts, and may cause serious trouble to the expected development of the explant if its basic requirements are not met (Karasawa et al., 2002). The agar is recognized for its gelling action; therefore, it is used to give support to the plant when it is placed in the culture medium. The agar may also be a controller of vitrification and hyper-hydration phenomena (Williams and Leopold, 1989).

The pH is another critical and very important factor for the culture medium as it influences both the availability of nutrients and phytoregulators and the degree of agar solidification. If the pH is well adjusted, it may promote a higher and better use of the nutrients by the explant. Therefore, the cells and tissues of plants require an appropriate pH range for the growth and development *in vitro*. Thus, the pH of the culture media are generally adjusted to 5.6 to 5.8 because in these conditions all ions are in solution and readily available for the cells.

Furthermore, it is a pH value close to those, which under natural conditions involve plant cells (Bhatia and Ashwath, 2005; Canhoto, 2010).

The oxidation is another important factor for the *in vitro* culture, this process is caused by the reaction of polyphenoloxidases on phenolic compounds, and may cause the death of the stem apexes in the early stages of development, or it may affect the performance of the multiplication phase (Souza et al., 2000). To reduce the phenolic oxidation, some procedures may be adopted, such as the use of antioxidant substances, reduce the mechanical and chemical damages, wash the vegetative propagules under running water, use more diluted basic media, remove the phenolic substances, among others (Xavier et al., 2009).

With regards to the antioxidant effect, it consists of the inactivation of free radicals, complexation of metabolic ions, or the reduction of peroxides for products unable to form free radicals with oxidative potential (Araújo, 1985). Among the substances with antioxidant effects, we may mention the ascorbic acid, citric acid, polyvinylpyrrolidone (PVP), activated carbon, L-cysteine, dithiothreitol, thiourea, coconut water, and bovine serum albumin. These substances may act by inhibiting the synthesis or action of enzymes related to the oxidation of polyphenols, or act as adsorbents of these substances (Goulart et al., 2010).

In this context, this study aimed to adjust a culture medium that meets the nutritional needs for the *in vitro* development of caçari, evaluating the effect of different concentrations, culture media, antioxidants, and agar and pH levels.

MATERIALS AND METHODS

The experiment was performed at the Tissue Culture Laboratory of Embrapa Roraima, in Boa Vista (Roraima State, Brazil). Stem segments with 4 pairs of axillary buds (± 6 cm length) from matrix plants of caçari with good phytosanitary health were used; these plants were kept in a greenhouse under daily irrigation, nutrition, and health care. Before obtaining the explants, the plants were pruned to induce new sprouting; then, they were pulverized with 2 $ml.L^{-1}$ fungicide (Nativo®) for 30 days, with an interval of seven days between one application and another.

After collection, the segments were taken to the laboratory and were submitted to a pre-cleaning process, excising the leaves and stem excess, in order to obtain a stem with ±1 to 1.5 cm with a pair of buds and ready to be inoculated, after the disinfestation process. Then, they were washed in running water for partial leaching of phenolic compounds released as a result of the tissue cutting. Afterward, they stayed for 24 h immersed in a solution composed of a mixture of $ml.L^{-1}$ fungicidal (Nativo®) and $2ml.L^{-1}$ bactericide (Kasumin®). Subsequently, the explants were taken to an inoculation room and disinfested in a laminar flow hood, immersed in a solution of alcohol (70%) for 1 min and, after that, they were immersed in sodium hypochlorite (1.5%) for 12 min, followed by three washes in distilled, deionized and autoclaved water (DDA water) for a complete removal of the products from the surface of the tissues.

After disinfestation, the explants were inoculated in 15 × 125 mm test tubes containing 30 mL culture medium, according to each experiment and treatments, described as follows.

Determination of the culture medium and concentration for the *in vitro* regeneration of caçari

After disinfestation, the explants were cultured in different media (WPM, MS and JADS), combined with four concentrations (25, 50, 75 and 100%) of the composition of mineral salts present in the culture media; 3 $g.L^{-1}$ activated carbon were added to all treatments, solidified with 7 $g.L^{-1}$ agar, and the pH was adjusted to 5.8, before autoclaving at 121°C and 1 atm for 20 min.

The experimental design was completely randomized in a 3×4 factorial scheme with five replications and five explants per replication were used for each treatment.

Influence of pH and agar concentration in the *in vitro* regeneration of caçari

The explants were cultured in WPM medium with different agar concentrations (0, 3.5, 7.0 and 10.5 $g.L^{-1}$), combined with different pH values (3.7, 4.7, 5.7 and 6.7), added with 3 $g.L^{-1}$ activated carbon, and autoclaved at 121°C and 1 atm for 20 min, before the pH adjustment.

The experimental design was completely randomized in a 4×4 factorial scheme with five replications and five explants per replication were used for each treatment.

Effect of different antioxidants and concentrations on the control of phenolic oxidation in stem segments of caçari

The explants were cultured in WPM medium containing different antioxidants (ascorbic acid (AA), citric acid (CA), polyvinylpyrrolidone (PVP)), combined with their concentrations (0, 100, 200, 300 $mg.L^{-1}$). It was used the WPM as a standard medium, with 4 $g.L^{-1}$ activated carbon, solidified with 7 $g.L^{-1}$ agar and pH adjusted to 5.8, before autoclaving at 121°C and 1 atm for 20 min.

The experimental design was completely randomized in a 3×4 factorial scheme with five replications and five explants per replication were used for each treatment.

For all experiments, after inoculation, the explants stayed for 15 days in darkness; then, they were transferred to a growth chamber and submitted to 16 h of photoperiod, at a temperature of 25 ± 2°C and luminosity of 32 $\mu mol.m^{-2}.s^{-1}$. After 90 days, the number and length of sprouts, and the oxidation were evaluated.

The results of the variables evaluated were submitted to the variance analysis by the statistic program Sisvar (Ferreira, 2011), performing the regression analysis for the quantitative factor and Tukey's test for the qualitative factor, at 5% of probability.

RESULTS AND DISCUSSION

Optimum culture medium and concentration for the *in vitro* regeneration of caçari

The analysis of variance showed an interaction between the culture media and concentrations established in this study for the sprout length and oxidation value. For the number of sprouts, there was a significant difference just for the culture media.

A greater number of sprouts were obtained in the JADS medium with a mean of 2.70 sprouts, followed by the MS medium (2.15) which did not significantly differ from JADS medium. The WPM medium provided a lower number of sprouts, but it did not differ from MS medium (Table 1). Similar results were found by other authors, who found that the JADS and MS media provided a sprout rate of 5.3 and 4.9, respectively, for the cultivation of *Eucalyptus* clones (Borges et al., 2011; Andrade et al., 2006). These two media provided similar increments in the number of sprouts in *Eucalyptus* clones. A similar behavior was observed for the number of sprouts obtained in our study.

Table 1. Number of sprouts from stem segments of caçari cultured *in vitro* using different culture media.

Culture media	Number of sprouts
WPM	1.68^{b}
JADS	2.70^{a}
MS	2.15^{ab}
CV (%)	45.5

Means followed by the same letter in the column do not differ from another by the Tukey test level of 5% probability.

The MS is a medium with a high content of nutrients (Leifert et al., 1995) and the JADS medium has a more diluted nutrient content in comparison to the MS medium. Therefore, by comparing the MS, JADS, and WPM media, it was observed that the MS and JADS have higher concentrations of macro and micronutrients than the WPM medium (Borges et al., 2011). Thus, there is an intimate correlation between the MS and JADS media; this fact may explain the best results regarding the number of sprouts by using these two media. Therefore, the nutritional similarity may have influenced the results, which showed no significant difference between the MS and JADS media.

Leitzke et al. (2010), studying the mulberry Xavante (*Rubus* sp.), also observed a higher number of sprouts using the MS medium (2.5 sprouts). A similar result was also obtained by Villa et al. (2005), who found a higher number of sprouts for the MS medium.

The vegetative growth of the sprouts and oxidation of the explants, submitted to different culture media and concentrations, are shown in Figure 1A and B, respectively.

With regards to the length of sprouts (Figure 1A), there was an interaction between the culture media and salt concentrations. The WPM medium provided a linear growth of sprouts with the increase of salt concentrations in the medium, promoting a higher average growth of sprouts (2.15 cm). Regarding the MS medium, there was a reduction in the sprout length, up to the concentration of 57% salts, obtaining sprouts with a mean of 0.99 cm; above this concentration, there was an increase in the sprout length with the increase of salt concentration. However, for the JADS medium, an opposite behavior was observed. There was an increase in the sprout length with the increase of the concentration of salts up to 50%, obtaining sprouts with a mean of 0.92 cm, but above this concentration, there was a reduction in the

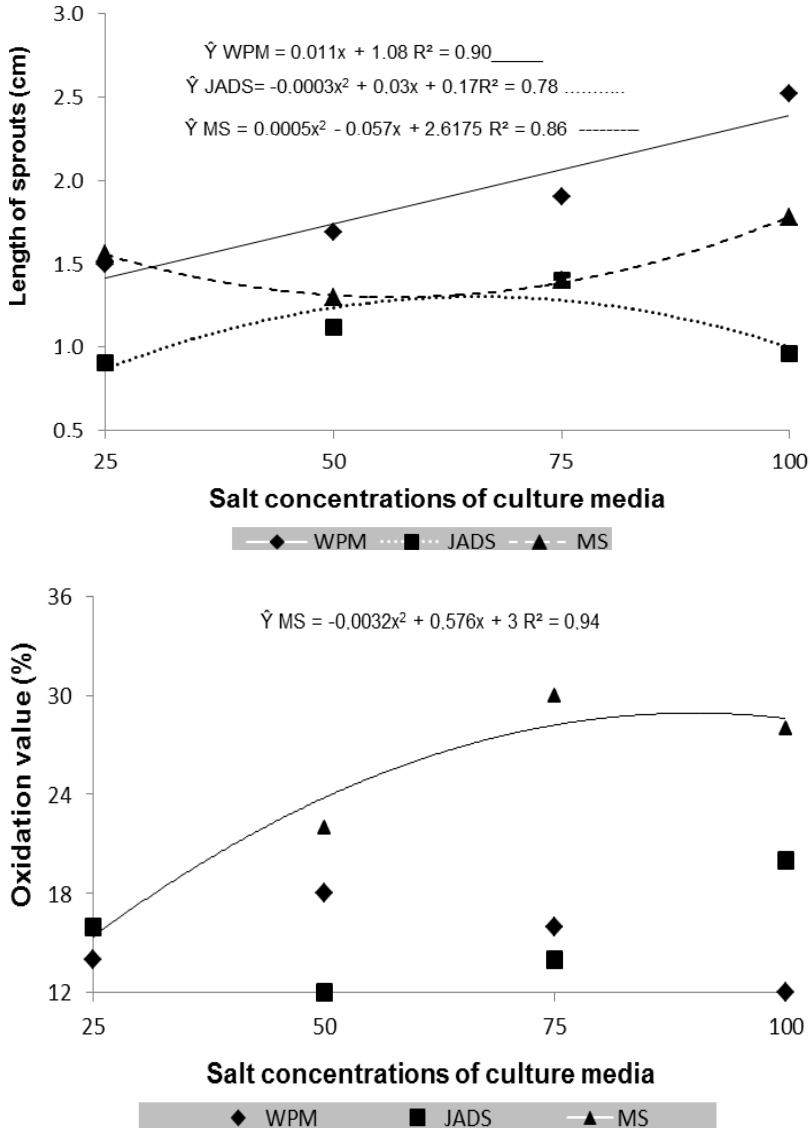

Figure 1. Length of sprouts (cm) (1A) and oxidation value (1B) of stem segments of caçari cultured in vitro in different culture media and salt concentrations.

sprout growth cultivated *in vitro*.

The WPM medium, in comparison to the MS and JADS media, has more diluted concentrations of macro and micronutrients. This medium has also been the most widely used in studies involving woody species, such as the caçari. Thus, probably, despite having provided a lower number of sprouts in comparison to the MS and JADS media (Table 1), the WPM provided a higher growth rate (Figure 1A). Therefore, if the aim is the *in vitro* propagation of a species, the obtainment of longer and more vigorous sprouts is better than a high number of small sprouts with lower capacity to regenerate or retard the multiplication process. It is noteworthy that for the production of conventional seedlings, the caçari is a rustic species and grows best in nutritionally poor substrates (Chagas et al., 2013). It is also evidenced that under the *in vitro* conditions, the best result for sprout length was obtained by using the culture medium with lower concentrations of nutrients, particularly the nitrogen and potassium.

Other authors also reported satisfactory results studying the *in vitro* culture with WPM for *Ficus carica* (fig tree), a woody plant. These authors found that the WPM medium promoted an optimal growth, reaching 6 cm length (Brum et al., 2002; Palú et al., 2014). Several studies have confirmed the efficiency of WPM in the *in vitro* culture of woody species using nodal segments and apical buds, such as *Eugenia involucrata* (Golle et al., 2012), *Tectona grandis* L. (Fermino Júnior et al., 2014), and coffee tree (*Coffea arabica*) (Rezende et al., 2008;

Jesus et al., 2010).

With regards to the oxidation (Figure 1B), after the interaction unfolding, we observed that only the MS medium and its salt concentrations showed a significant difference. Regarding the WPM and JADS media, there was no significant difference, so in Figure 1B, we did not insert the trend line at the curve points of these media.

For the MS medium, the lowest oxidation value was observed at the concentration of 25%, however, as the increase of the salt concentrations up to 90%, there was a higher oxidation (29%). For the WPM and JADS, a total mean of 12 and 15.5% of oxidized explants, respectively was verified, with no influence of the salt concentrations since there was no significant difference between the media and concentrations (Figure 1B). Therefore, it is noteworthy that the three media showed low oxidation.

Different results were found by Fagundes et al. (2012) studying stem explants of *Campomanesia guazumifolia* (Myrtaceae). These authors found that the WPM medium led to a higher oxidation rate in comparison to the MS medium (98.33 and 92.50%, respectively). According to Teixeira (2001), some species of plants are more susceptible to oxidation than other species. Golle et al. (2012), studying the *Eugenia involucrata,* also observed an oxidation process, however, these processes did not affect the establishment and development of the cultures.

Similar results to those presented in this study were found in other studies. Pelegrini et al. (2013), studying zygotic embryonic axis of a forest species (*Ocotea porosa*), found that the MS medium promoted a higher oxidation (80%), in comparison to the WPM (30%). For other forest species, these authors, studying the axillary buds of "jacaranda da Bahia", cultured in MS and WPM, found no significant differences between these two media, finding a mean of 70% of oxidation, for both MS and WPM (Sartor et al., 2013). Thus, the oxidation values found in this study is lower than those found by other authors. Therefore, it was observed that the lowest oxidation values were obtained for the WPM and JADS media, with a mean of 12 and 15% of oxidation, respectively, even at a concentration of 100% salts, evidencing that the WPM favored the *in vitro* regeneration process of caçari, probably due to provide the best nutritional balance for this species.

Influence of pH and agar concentration in the *in vitro* regeneration of caçari

There was a significant interaction between the factors tested for the number and length of sprouts. With regards to the oxidation, there was a significant difference only for the agar factor. Figure 2 show the number (Figure 2A) and length (Figure 2B) of sprouts. Best results were observed for the concentration of 7 $g.L^{-1}$ of agar. At this concentration, there was higher number of sprouts with the increase of the pH of the culture medium, up to the concentration of 6.04, obtaining 7.6 sprouts. This probably occurred because the concentration of 7 $g.L^{-1}$ promotes a support and availability of suitable nutrients for the growth of the plant since it is the agar concentration most commonly used for most species cultured *in vitro*. At the concentration of 3.5 $g.L^{-1}$, there was a linear increase in the number of sprouts as the increase of the pH of the culture medium, resulting in better means when combined with a pH of 6.7. However, for the concentration equal to zero, there was a different behavior in comparison to the previous concentrations since there was a decrease in the number of sprouts as the increase of the medium pH. Regarding the concentration of 10.5 $g.L^{-1}$ agar, there was no significant difference. This occurred because a higher concentration of agar (10.5 $g.L^{-1}$) provides a stiffer medium and hinders the absorption of nutrients and development of the sprouts.

Similar results to those obtained in this experiment were observed by Pereira (2014), who evaluated the pH and nitrogen source effects in the *in vitro* propagation of "medonheiro" (*Arbutus unedo* L.), finding a preference for pH close to neutrality (5.7 to 6.5) for the proliferation of sprouts. Karim et al. (2007), evaluating the effect of sucrose concentration and pH levels in the *in vitro* regeneration of *Araria elata*, observed a higher number of sprouts in pH ranging from 4.5 to 5.8, thus corroborating the results obtained in the present study. Nair and Seeni (2003) also observed a best multiplication rate of *Calophyllum apetalum* with the medium pH adjusted to 5.8.

However, different results were found by Naik et al. (2010), studying the *Bacopa monnieri*. These authors obtained a higher number of sprouts in more acidified culture media, and the best results were observed at pH of 4.5, obtaining 151 sprouts. Similar results were reported by Bhatia and Ashwath (2005), who found the best sprout *in vitro* regeneration, for tomato (*Solanum lycopersicum* L.), using media with more acidified pH, in comparison to more alkaline pH media.

With regards to the length of sprouts (Figure 2B), there was a significant interaction only for the concentrations of 3.5 and 7 $g.L^{-1}$ agar and pH levels. For the concentration of 3.5 $g.L^{-1}$ agar, there was a linear increase in the sprout length as the increase of the pH levels. For the concentration of 7 $g.L^{-1}$ agar, there was an increase in the growth of sprouts as the increase of the medium pH up to 4.95, resulting in a sprout growth of 4.43 cm. Regarding the concentrations of 3.5 and 7.0 $g.L^{-1}$ agar, the higher the pH the higher the average growth of sprouts. For the concentrations of 0 and 10.5 $g.L^{-1}$ agar, the data were not significant, indicating that the caçari has a better development in culture media closer to neutrality than when it is cultured in an acidic medium with a pH below 4.5 since more acidic culture media hamper the availability of nutrients for the explant.

Karim et al. (2007), evaluating the sucrose

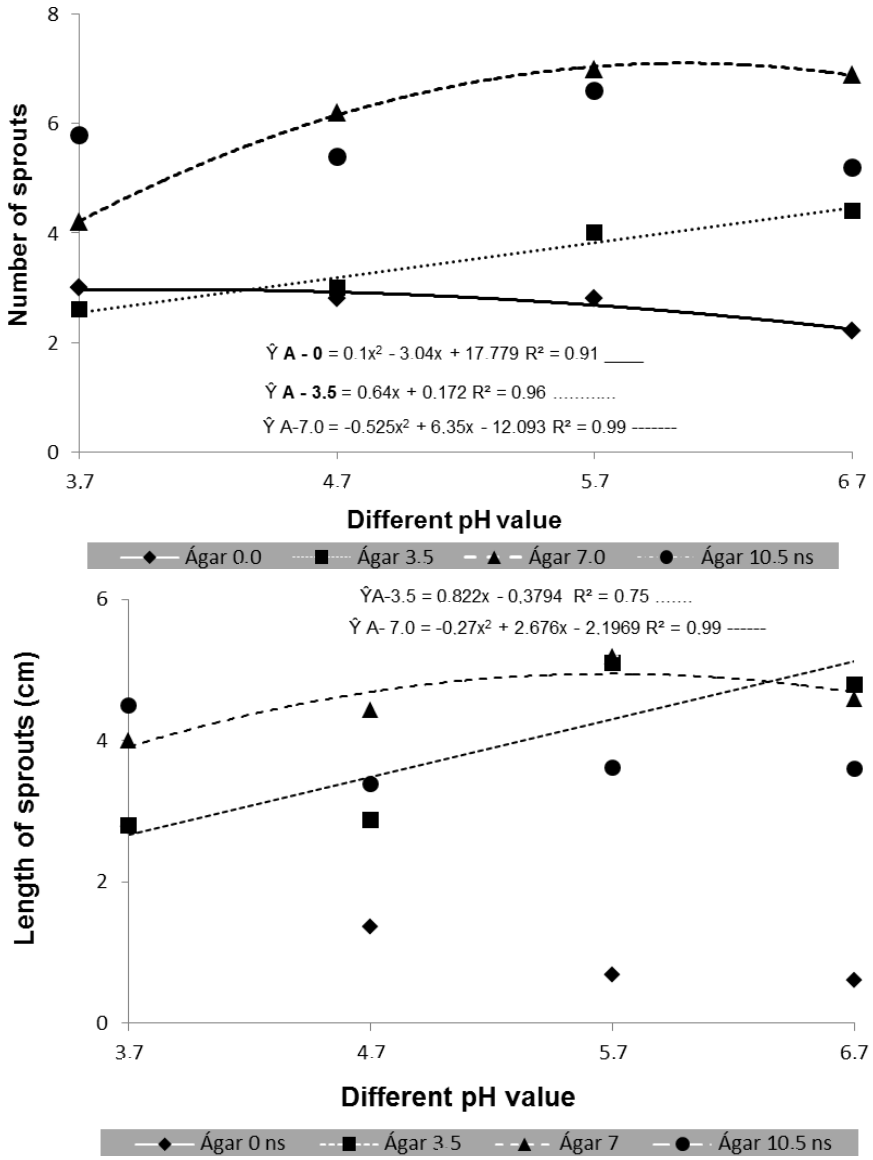

Figure 2. pH and agar effect on the number (A) and length (B) of caçari sprouts cultured *in vitro*.

concentration effect and pH levels on the *in vitro* regeneration of *Araria elata*, also observed a higher sprout growth at pH of 5.8, obtaining sprouts of 5.9 cm. However, pH above 5.8 significantly reduced the development of the sprouts *in vitro*. On the other hand, Pasqual et al. (2002) observed a greater height of the aerial part (2.3 cm) in tangelo tangerine (*Citrus reticulata.*), with pH adjusted to 4.7 and 9.3 $g.L^{-1}$ agar. Subsequently, there was a decrease in this variable as the increase of agar concentration.

Suthar et al. (2011), evaluating the influence of agar on the sprouting and sprout length of *Boswellia serrata*, observed better results in liquid culture medium and low agar concentrations; these results differ from those observed in the present study, wherein the best results were obtained in the concentration of 7 $g.L^{-1}$ agar.

Effect of different antioxidants and concentrations in the control of phenolic oxidation in stem segments of caçari

There was a significant difference in the interaction between the antioxidants and concentrations used for all analyzed variables.

Figure 3 shows the vegetative growth through the variables number (Figure 3A) and length (Figure 3B) of sprouts.

Regarding the number of sprouts, there was no significant effect of the citric acid (antioxidant), therefore,

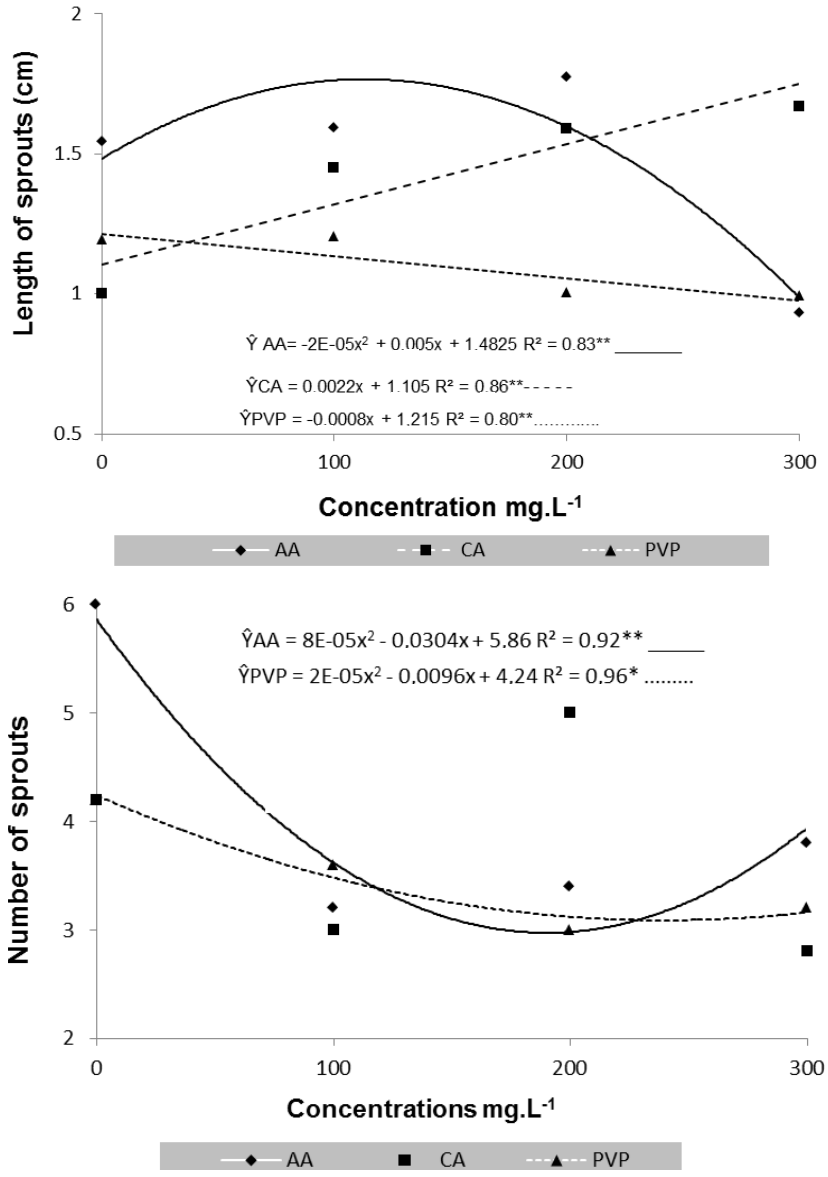

Figure 3. Effect of ascorbic acid, citric acid, and polyvinylpyrrolidone (PVP), combined with different concentrations, on the number (A) and growth of sprouts (B) of stem segments of caçari cultured *in vitro*.

we did not insert a trend line for it, exposing only the sprouting number mean. However, for the ascorbic acid and PVP, a similar behavior was found; there was a decrease in the number of sprouts as the increased of these antioxidant concentrations, in the culture medium, up to the concentration of 190 and 240 $mg.L^{-1}$, obtaining means of 2.97 and 3.08 sprouts, respectively. From these concentrations, there was a small increase in the number of new sprouts. However, the highest values (6 and 4.2 sprouts) for this variable were observed in the absence of the tested antioxidants (Figure 3A). Probably, the high antioxidant concentrations were toxic to the explants, so the number of explant sprouts was affected.

With regards to the sprout number, different results from those presented herein were found in a study on explants of paricá (*Schizolobium amazonicum*), in which the use of PVP (concentration of 0.2%) promoted a higher mean of sprouts (Cordeiro et al., 2014). On the other hand, Souza et al. (2014), testing different citric acid and ascorbic acid concentrations for the *in vitro* culture of *Schomburgkia crispa*, observed that a higher number of sprouts were obtained in the absence of antioxidants. Camolesi et al. (2007) also observed that the absence of antioxidant provided a higher number of sprouts in banana apexes (*Musa* spp.). Thus, it was found that the antioxidant effect, observed for the number

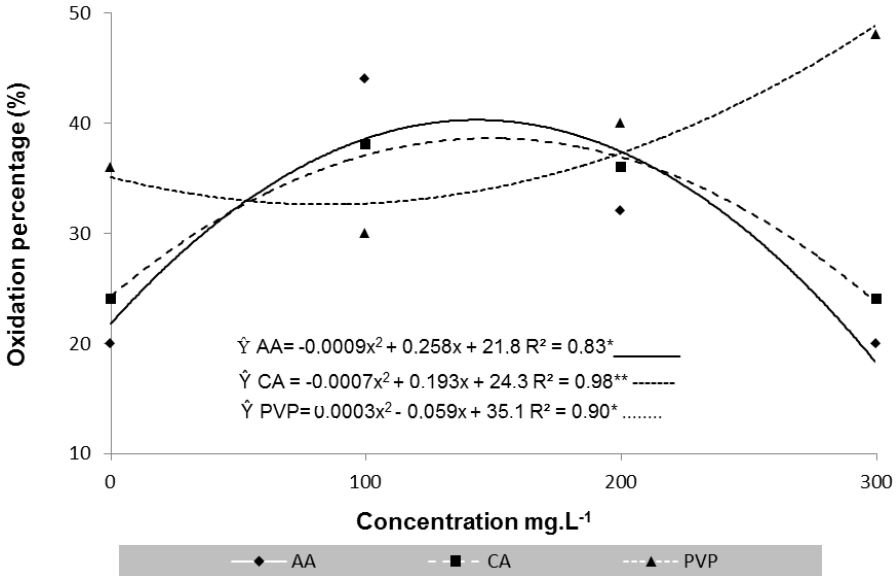

Figure 4. Effect of ascorbic acid, citric acid, polyvinylpyrrolidone, and different concentration, on the oxidation control of caçari stem segments cultured *in vitro*.

of explant sprouts of caçari cultured *in vitro* with different antioxidants and concentrations, is similar to those observed in explants of banana and *S. crispa*.

Concerning the sprout length, there were different behaviors for each antioxidant and concentrations (Figure 3B). For the ascorbic acid (AA), there was a great sprout growth (1.8 cm) up to the concentration of 125 $mg.L^{-1}$; above this concentration, there was a decrease in the sprout length. In the citric acid (CA), there was a linear sprout growth with the increase of this antioxidant concentrations, evidencing that, for this antioxidant, adjustments are needed to find an optimal concentration. For the PVP, there was an opposite behavior in comparison to the CA, with a decrease in the sprout length with the increase of the concentrations in the culture medium, and the higher sprout length was observed in the absence of an antioxidant. Probably it occurred because of the used material, given that the explants used *in vitro* are extremely small structures, which are very susceptible to dehydration and rapid oxidation since they belong to a woody and recalcitrant species. They may also exhibit different behaviors when submitted to treatment with high concentrations of antioxidants.

Camolesi et al. (2007) observed that the absence of antioxidant provided a greater growth of sprouts, in banana apexes. Similar results were reported by Souza et al. (2014), who tested different concentrations of citric acid and ascorbic acid in *S. crispa* Lindl, and found that a greater sprout length was observed in the absence of antioxidants.

For the oxidation, the use of AA antioxidant resulted in the oxidation increase, up to the concentration of 143.33 $mg.L^{-1}$, obtaining 40.2% of oxidation. For the CA antioxidant, there was a similar behavior, with an increase of the oxidation, up to the concentration of 138.1 $mg.L^{-1}$; resulting in 37.6% of oxidized explants. Whereas in the absence of these two types of antioxidants, there was a lower oxidation: 20% (AA) and 24% (CA). For the PVP, there was a decrease in the oxidation (38%), up to the concentration of 98 $mg.L^{-1}$; from this concentration, the oxidation rate tended to increase (Figure 4). The PVP was less effective to control the oxidation of caçari explants in comparison to the citric acid and ascorbic acid. This probably occurred by the fact the PVP may adsorb the phenolic compounds produced by the plant; whereas the CA and AA are reducing agents of oxidative enzymes, reducing the production of toxic substances.

Different from the results obtained in the present experiment, other studies were performed aimed to evaluate the PVP effect on the oxidation control of paricá (*Schizolobium amazonicum*) explants, in which the addition of 0.2 and 0.3% PVP, in the culture medium, controlled 100% of the oxidation, and the lowest value of PVP (0.1%) controlled only 80% of the oxidation. This effect shows the importance of antioxidants in the culture medium to control this limiting factor in micropropagation (Oliveira et al., 2011; Cordeiro et al., 2014).

In banana explants, it was observed that the pretreatment only immersing the explants in solution with antioxidant was enough to control the oxidation. However, by adding this antioxidant in the culture medium, the oxidation increased (Camolesi et al., 2007). This procedure is recommended by Anthony et al. (2004) and Grattapaglia and Machado (1998), who confirmed the results obtained for the banana. For the

Symonanthus bancroftii (Panaia et al., 2000) and *Conostephium pendulum* (Anthony et al., 2004), these authors described that the combination of 0.25 g L^{-1} citric acid with 0.75 g.L^{-1} potassium citrate, used in pretreatment and added to the culture medium, reduced the necrosis of the excised tissue, in addition to preventing the oxidation process.

Werner et al. (2009) tested some antioxidants such as ascorbic acid, citric acid, and activated carbon, in the calogenesis of *Caesalpinia echinata*. The activated carbon showed a better oxidation control, wherein 40% of the explants showed an oxidation ranging from low to moderate, but without callus formation, demonstrating that still there are factors that have to be studied, such as the addition of activated carbon to the culture medium and pretreatment of the tissues using antioxidant agents, instead of adding to the culture medium.

Conclusions

For the *in vitro* regeneration of caçari, the best results are obtained using the WPM medium at a concentration of 100% with 7 g.L^{-1} agar, and pH adjusted to 5.7. The use of antioxidants in the tested conditions did not contribute to decrease in oxidation in explants, indicating that there is no need of adding them into the culture medium.

Conflict of interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors thank the Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES) for scholarship granted to the first author, from Strategic Programs, No: BEX 2724 / 14-6. They express their thanks to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and scientific research and the State of the Environment Foundation Environment and Water Resources (FEMARH) for financial support and Embrapa and the University of Florida for the opportunity for the realization of this experiment.

Abbreviations

WPM, Woody plant medium; **MS,** Murashige and Skoog medium; **AA,** ascorbic acid; **CA,** citric acid; **PVP,** polyvinylpyrrolidone, **JADS,** Juan, Antonio, Diva and Silvia medium.

REFERENCES

Andrade WF, Almeida M, Gonçalves NA (2006). *In vitro* multiplication of *Eucalyptus grandis* under benzylaminopurine stimuli. Pesq. Agropec. Bras. 41(12):1715-1719.

Anthony JM, Senaratna T, Dixon KW, Sivasithamparam K (2004). The role of antioxidants for initiation of somatic embryos with *Conostephium pendulum* Benth. (Ericaceae). Plant Cell Tissue Organ. Cult. 78(3):247-252.

Araújo JMA (1985). Food chemistry: theory and practice. Viçosa, MG: Universidade Federal de Viçosa. 355p.

Bhatia P, Ashwath N (2005). Effect of medium pH on shoot regeneration from the cotyledonary explants of Tomato. Biotechnology 4:7-10.

Borges SR, Xavier A, Oliveira LS, Lopes AP, Otoni WC (2011). *In vitro* multiplication of *Eucalyptus globulus* hybrid clones. Rev. Árv. 35(2):173-182.

Brum GR, Silva AB, Pasqual M (2002). Effect of different concentrations of BAP and ANA in *in vitro* propagation of fig tree (*Ficus carica* L.). Ciênc. Agrotecnol. Edição Especial. pp. 1403-1409.

Camolesi MR, Kaihara ES, Saconi CG, Faria RT, Neves CSVJ (2007). Oxidation reduction in *in vitro* propagation of 'apple' banana. Ciênc. Agrotec. 31(4):1237-1241.

Canhoto JM (2010). Plant Biotechnology of Plant Cloning to Genetic Transformation. Imprensa da Universidade de Coimbra. Coimbra. 407p. Available at: https://digitalis.uc.pt/files/previews/56563_preview.pdf

Chagas EA, Lima CGB, Carvalho AS, Ribeiro MIG, Sakazaki RT, Neves LC (2012). Camu-camu propagation (*Myrciaria dubia* (H.B.K.) Mcvaugh). Agroamb. 6: 67-73.

Chagas EA, Lozano RMB, Chagas CP, Bacelar-Lima CG, Garcia MIR, Oliveira JV, Souza OM, Morais BS, Araújo MCR (2015). Intraspecific variability of camu-camu fruits in native populations in northern Amazonia Forest. Crop Breed. Appl. Biotechnol. 15(4):265-271.

Chagas EA, Ribeiro MIR, Souza OM de, Santos VA dos, Lozano RMB, Bacelar-Lima CG (2013). Alternative substrates for the production of camu-camu seedlings. Rev. Ciênc. Agric. 56:1-7.

Cordeiro MNS, Lemos OF, Rodrigues SM, Menezes IC (2014). *In vitro* control of shoots oxidation and induction in stem apex of Schizolobium *Schizolobium amazonicum* Huber ex Ducke. In. 18th Seminar of Scientific Iniciation and 2nd Post-graduation seminar of Embrapa Eastern Amazonia, Annals. pp. 1-5.

Correia D (2006). Macronutrients, nutritional and biochemical aspects in *in vitro* growth of *Eucalyptus grandis* shoots. Theses (Doctorate in Natural resources). Universidade de São Paulo Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba, 2006. 176p.

Correia D, Gonçalves NA, Couto HTZ, Ribeiro MC (1995). Efeito do meio de cultura líquido e sólido no crescimento e desenvolvimento de gemas de *Eucalyptus grandis* X *Eucalyptus urophylla* na multiplicação *in vitro*. IPEF. 48/49:107-116.

Fagundes LS, Bernardy K, Koefender J, Golle DP (2012). A study on the *in vitro* establishment of (*campomanesi aguazumifolia* [cambess.] O. Berg) - Myrtaceae. In: XVII Inter-institutional Seminar on Teaching, Research and Extension. Rio Grande do Sul. Annals Rio Grande do Sul: UNICRUZ. P 4.

Fermino Junior PCP, Raposo A, Nagao EO, Scherwinski-Pereira JE (2014). Effects of different cytokines and double-phase culture system in the teak micropropagation (*Tectona grandis* L.) established in South-Western Amazon. Evidência 14(1):7-20.

Ferreira DF (2011). Sisvar: A computer statistical analysis system. Ciênc. Agrotecnol. 35(6):1039-1042.

Golle DP, Reiniger LRS, Curti AR, León EAB (2012). *In vitro* establishment and Development of *Eugenia involucrate* DC: Influence of explant type and nutrient medium. Ciênc. Florest. 22(1):207-214.

Goulart PB, Xavier A, Dias JMM (2010). Effect of antioxidants on the rooting of mini-cuttings of *Eucalyptus grandis* x *E. urophylla* clones. Rev. Árv. 34(6):961-972.

Grattapaglia D, Machado MA (1998). Micropropagation. In. Torres AC, Caldas LS, Buso JA, Tissue culture and genetic transformation of plants. Brasília, DF: Embrapa-SPI. 1:183-260.

Jesus MAS, Carvalho SP, Villa F, Pasqual M, Carvalho M (2010). *In vitro* development of coffee shoots in different culture media and plant growth regulators. Sci. Agra. 11(1):431-436.

Karasawa MMG, Pinto JEBP, Pinto JC, Pereira AV (2002). The influence of growth regulator and medium on proliferation of *in vitro*

propagated elephant grass. Ciênc. Agrotecnol. 26:1243-1251.
Karim MZ, Yokota S, Rahman MM, Eizawa J, Saito Y, Azad MAK, Ishiguri F, Iizuka K, Yoshizawa N (2007). Effect of sucrose concentrations and pH level on shoot regeneration from callus in *Aralia elata* Seem. Asian J. Plant Sci. 6:715-717.
Leifert C, Murphy KP, Lumsden PJ (1995). Mineral and carbohidraate nutition of plant cell and tissue cultures. Crit. Rev. Plant Sci. 14(2):83-109.
Leitzke LN, Damiani CR, Schuch MW (2010). The influence of culture medium, cytokinin type and concentrations on *in vitro* multiplication of blackberry and raspberry. Ciênc. Agrotecnol. 34(2):352-360.
Lloyd G, Mccown B (1981). Commercially feasible micropropagation of montaim laurel, Kalmia latifolia, by use of shoot tip culture. Combined Proceedings Internat. Plant Propagators Society 30:421-427.
Murashige T, Skoog F (1962). A revised medium for rapid growth and biossays with tobacco tissue cultures. Physiol. Plant. 15:473-479.
Naik PM, Manohar SH, Praveen N, Murthy HN (2010). Effects of sucrose and pH levels on *in vitro* shoot regeneration from leaf explants of *Bacopa monnieri* and accumulation of bacoside A in regenerated shoots. Plant Cell Tissue Org. Cult. 100:235-239.
Nair LG, Seeni S (2003). *In vitro* multiplication of *Calophyllum apetalum* (Clusiaceae) an endemic medicinal tree of the Western Ghats. Plant Cell Tissue Organ. Cult. 75:169-174.
Neves LC, Silva VX, Chagas EA, Barcelar-Lima CG, Roberto SR (2015). Determining the harvest time of camu-camu [*Myrciaria dubia* (H.B.K.) McVaugh] using measured pre-harvest attributes. Sci. Hortic. 186:15-23.
Oliveira HS, Lemos OF, Miranda VS, Moura HCP, Campelo MF, Santos LRR (2011). Establishment and *in vitro* multiplication of shoots in the micropropagation process of banana cultivars (Musa spp.). Acta Amaz. 41(3):369-376.
Palú EG, Krause S, Silva LM da, Hunhoff VL, Dalbosco EZ (2014). Nutrient media in *in vitro* establishment of fig. Enciclopédia Biosfera, Centro Cient. Conhecer. 10(19):1280-1286.
Panaia M, Senaratna T, Bunn E, Dixon KW, Sivasithamparam K (2000). Micropropagation of the critically endangered Western Australian species, *Symonanthus bancroftii* (F. Muell.) L. Haegi (Solanaceae). Plant Cell Tissue Organ. Cult. 63(1):23-29.
Pasqual M, Finotti DR, Dutra LF, Chagas EA, Ribeiro LO (2002). *In vitro* culture of 'poncã' mandarin immature embryos: ph x agar concentration. Rev. Bras. Agroc. 8(3):199-202.
Pelegrini LL, Ribas LLF, Zanette F, Koelher, HS (2013). *In vitro* germination of zygotic embryonic axes of imbuia (Ocotea porosa (Nees ex Martius) Liberato Barroso). Rev. Árv. 37(20):231-236.
Pereira RAG (2014). Effect of pH and of nitrogen source in *in vitro* culture of "medonheiro" (*Arbutus unedo* L.). Dissertation (Master's thesis in Biodiversity and Plant Biotechnology). Universidade de Coimbra. 84p. Available at: http://www.uc.pt/uid/medronheiro/Publicacoes/Tese_Mestrado_Rui_Pereira.
Rezende J, Pasqual M, Carvalho SP, Pereira AR, Villa F (2008). Influence of culture medium and agar concentration in the growth and development of coffee seedlings derivative from direct somatic embryogenesis. Sci. Agrar. 9(1):21-26.
Rocha SC (2005). Micropropagation of *Cabralea canjerana*. Master's thesis (Botany) - Department of Botany, Universidade Federal do Paraná, Curitiba, PR. Available at: http://www.bibliotecaflorestal.ufv.br:80/handle/123456789/17196
Sartor FR, Zanotti RF, Pôssa KF, Pilon AM, Fukushima CH (2013). Different culture media and antioxidants in *in vitro* establishment of 'jacaranda da Bahia. Biosci. J. 29(2):408-411.
Souza ANS, Silva SR da, Sorgato JC, Soares JS, Rosa YBCJ (2014). Antioxidants and absence of light in development *in vitro* of *Schomburgkia crispa* Lindl. Enciclopédia Biosfera. Centro Cient. Conhecer. 10(18):333-342.
Souza AS, Cordeiro ZJM, Trindade AV (2000). Seedling production. In. Cordeiro, Z. J. M. Banana: production. Brasília, DF: Embrapa Comunicação para Transferência de Tecnologia. pp. 39-46.
Suthar RK, Habibi N, Purohit SD (2011). Influence of agar concentration and liquid medium on *in vitro* propagation of *Boswellia serrate* Roxb. Indian J. Biotechnol. 10:224-227.
Teixeira JB (2001). Limitations in *in vitro* cultivation process of woody species. In. Encontrolatinoamericano de Biotecnologia Vegetal, 4.,2001, Goiânia, Simpósios. Goiânia: REDBIO
Villa F, Araújo AG de, Pio LAS, Pasqual M (2005). *In vitro* multiplication of blackberry 'Ébano' at different concentrations of MS and BAP medium. Ciênc. Agrotecnol. 29(3):582-589.
Werner ET, Cuzzuol GRF, Pessotti KV, Lopes FP, Roger JA (2009). *In vitro* calogenesis control of Pau Brasil. Rev. Árv. 33(6):987-996.
Williams RJ, Leopold AC (1989). The glassy state in corn embryos. Plant Physiol. 89:977-981.
Xavier A, Wendling I, Silva RL (2009). Clonal silvicultura: principles and techniques. Viçosa, MG: Universidade Federal de Viçosa. 272p.