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IN VITRO ESTABLISHMENT AND CALLOGENESIS IN SHOOT TIPS OF PEACH PALM[1]

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ABSTRACT - *Bactris gasipaes* is an important Amazonian culture as the main source of hearts of palm. Techniques of plant tissue culture are promising tools in breeding programs of this culture. The objective of this study was to develop protocols for the *in vitro* establishment and callus induction in *Bactris gasipaes* shoot tips. Shoots were collected from young plantlets of *B. gasipaes*, which were disinfected with NaOCl 0.63, 1.25 and 1.88% (v/v), for 10, 20 and 30 minutes. After that, shoot tips were removed and inoculated in MS medium with factorial combinations of the growth regulators 2,4-D (0.0; 5.0; 10.0; 20.0 and 40.0 mg.L^{-1}) and BA (0.0; 3.0 and 6.0 mg.L^{-1}). The experimental design was entirely randomized, replicated three times with ten tubes containing one explant per plot. The disinfection was efficient for 20 minutes of immersion in NaOCl 1.25%, which resulted in 90% of explants without contamination and low oxidation. The greater callogenesis percentage was of 60%, reached at 10.0 mg.L^{-1} 2,4-D and 3.0 mg.L^{-1} BA combination.

Keywords: *In vitro* culture. *Bactris gasipaes*. Amazon Forest.

ESTABELECIMENTO *IN VITRO* E CALOGÊNESE EM ÁPICES CAULINARES DE PUPUNHEIRA

RESUMO - A pupunha é uma importante cultura amazônica e destaca-se como a principal fonte produtora de palmito. Técnicas de cultura de tecidos vegetais são uma ferramenta promissora para os programas de melhoramento dessa cultura. O objetivo desse trabalho foi desenvolver protocolos para estabelecimento *in vitro* e indução de calos em ápices caulinares de pupunha. Perfilhos de plantas jovens de pupunheira passaram por testes de desinfestação com hipoclorito de sódio nas concentrações de 25, 50 e 75% (v/v), por 10, 20 e 30 minutos. Em seguida, foram retirados os ápices e inoculados em meio MS acrescido de combinações fatoriais dos reguladores de crescimento 2,4-D (0,0; 5,0; 10,0; 20,0 e 40,0 mg.L^{-1}) e BAP (0,0; 3,0 e 6,0 mg.L^{-1}). Os experimentos foram dispostos em delineamento inteiramente casualizado, com 3 repetições por tratamento, sendo cada repetição composta por 10 tubos de ensaio contendo um explante cada. A desinfestação foi mais efetiva combinando 20 minutos de imersão com a concentração de hipoclorito de sódio de 1,25%, resultando em 90% de explantes sem contaminação. A maior porcentagem de indução de calos foi de 60%, a qual foi obtida com a combinação de 10,0 mg.L^{-1} de 2,4-D e 3,0 mg.L^{-1} de BAP.

Palavras-chave: Cultivo *in vitro*. *Bactris gasipaes*. Floresta Amazônica.

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INTRODUCTION

The peach palm (*Bactris gasipaes* H.B.K.) is a domesticated species that can be cultivated as a sustainable and profitable culture, producing hearts of palms every year (TOZETTI; CONTEL, 2003), taking the place of *Euterpe edulis* Mart. in the West and *Euterpe oleracea* Mart. in the North of Brazil (CARVALHO; ISHIDA, 2002), which natural reserves are already very reduced by predatory exploitation (VILLACHICA, 1996). The hearts of palms from the peach palm have a commercial advantage because it does not oxidate after cutting, which makes the industrial process easier and cheaper than the other palms (GUERREIRO, 2002).

B. gasipaes can be propagated by seeds, which take 30 to 90 days to germinate (VILLACHICA, 1996). According to Mora-Urpi et al. (1984), this kind of propagation is not much efficient due to self incompatibility, being the xenogamy the predominant form of reproduction of this species. Other restrictive aspects for the reproduction by seeds are the long time taken to produce hibrid seeds and the limited cross polinization in the beginning of the flower induction, which results in the reduction of fruit production and formation of partenocarpic fruits.

The propagation also occurs by shoots, which can be found in a number of about five per plant. This form of propagation is very slow and most of times cannot be useful to breeding programs whatever products we want (hearts of palms or fruit). The limited number of shoots prevents the formation of great populations genetically identical to the selected plant, which makes inefficient the breeding programs based in this method (ALMEIDA; ALMEIDA, 2006; SOUZA et al., 1996).

In this context, methods of *in vitro* propagation can be very useful as promising tools to subsidize breeding programs, allowing the clonal propagation of plants in large scale with high phytosanitary quality and uniformity. These techniques are able to clone determined plant that has several interesting characteristics to the breeding whithout the problems from the traditional propagation methods (BELTRÃO et al., 2008; GRATTAPAGLIA; MACHADO, 1998; RÊGO et al., 2009; SOARES et al., 2009; TORRES et al., 2001; TORRES et al., 1998a).

The propagation by tissue culture can be direct or indirect, the last one by callus formation and the return to the meristematic level from differentiated cells and then the transformation into new shoots and plants, which is a potential mass propagation (LANDA et al., 2000).

Studies indicate the existence of differences in morphogenic responses regarding age of explants employees (FIRMINO JÚNIOR. et al., 2009), emphazing the juvenile tissues, whose property is related to its higher meristematic activity (PEREIRA et al., 2000; BECERRA et al., 2004).

Conditions for callus formation and growing must be studied. The cultivation of callus can result in shoots or somatic embryos production, being needed the exogenous supplement of growth regulators is needed. The hormonal balance between auxins and citokinins is the most important aspect for callus culture (NOGUEIRA et al., 2007), with distinction for 2,4-D and, more recently, TDZ (AKRAM; AFTAB, 2008).

This study had as objective to develop protocols of *in vitro* establishment and to evaluate the callus induction in *B. gasipaes* shoot tips to make available information that can be useful in future breeding programs.

MATERIAL AND METHODS

The experiments took place at the Plant Tissue Culture Laboratory of the Brazilian Agricultural Research Corporation (Empresa Brasileira de Pesquisa Agropecuária) – Rondônia Branch, in Porto Velho.

Shoots of about 50 cm long were selected and collected from 3 m long young plants from the experimental field. At the laboratory, the shoots were washed and cut in 125 cm^3 (5 x 5 x 5 cm) parts, which contained the meristematic zone. These fragments were washed with water and a detergent agent and immersed in NaOCl 0.63, 1.25 and 1.88% during 10, 20 and 30 minutes and washed three times in sterile distilled water. After that, the fragments were cut into shoot tips of about 0.5 cm long. Then, each shoot tip was longitudinally cut and inoculated in assay tubes with MS medium (MURASHIGE; SKOOG, 1962) with the surface cut in contact with the medium. This medium was supplemented with sucrose 30.0 $g.L^{-1}$ and agar 8.0 $g.L^{-1}$, pH 5.8, and factorial combinations of 2,4-D (0.0; 5.0; 10.0; 20.0 and 40.0 $mg.L^{-1}$) and BA (0.0; 3.0 and 6.0 $mg.L^{-1}$). The tubes were maintened in the darkness in a growth-chamber at 25±2°C for 36 days. The experimental design was entirely randomized, replicated three times with ten tubes containing one explant per plot.

At the 10^{th} day, the contamination, necrosis and oxidation of the explants were evaluated and, at the 36^{th} day, the presence of callus in the explants was observed.

Statistical analyses were performed by Tukey test, at a 5% probability level.

RESULTS AND DISCUSSION

The most efficient disinfection treatment was the 20 minutes of immersion in NaOCl 1.25%, which resulted in 90% of explants without contamination

and 30% of oxidation (Table 1). The contamination was higher in the other treatments, reaching 50% with immersion in NaOCl 0.63% for 10 minutes. According to Teixeira (2009), the disinfection of shoots, leaves and buds can be successful utilizing etilic alcohol from 50 to 70% for 1 to 3 minutes, followed by NaOCl from 0.5 to 2.0% (w/v) for 5 to 20 minutes.

Table 1. Contamination and oxidation of *B. gasipaes* shoot tips in relation to time of immersion and concentration of NaOCl.

Time (min.)	Concentration (%)	Contamination (%)	Oxidation (%)
10	0,63	50[a]	20[d]
20	1,25	40[ab]	30[cd]
30	1,88	40[ab]	30[cd]
10	0,63	30[b]	30[cd]
20	1,25	10[c]	30[cd]
30	1,88	30[b]	50[ab]
10	0,63	30[b]	40[bc]
20	1,25	40[ab]	50[ab]
30	1,88	40[ab]	60[a]

*Means followed by the same letter are not significantly different ($p<0.05$).

A positive relation between the time of exposition to NaOCl, its concentration and the percentage of explant oxidation was observed. This oxidation was followed by tissue necrosis. An efficient system of disinfection is the one that matches low contamination and oxidation of the explants, with the lowest exposition possible to the disinfection agent (TORRES et al., 1998).

Callus induction in the explants was not observed in the absence of growth regulators (Figure 1). In other treatments, the induction process started with the swelling of explants, at the 10th day of culture.

At the 36th day, the need of 2,4-D for callus induction was observed (Figure 1). The induction increased with the concentrations of 2,4-D until 5.0 or 10.0 mg.L^{-1} 2,4-D, decreasing after that. The greater callogenesis percentage was of 60%, reached at 10.0 mg.L^{-1} 2,4-D and 3.0 mg.L^{-1} BA combination. The same concentration of 2,4-D, without BA, results in 30% of induction. In this work, callus obtained were white and compact.

The effect of BA was weaker. In the absence of this regulator callogenesis reached 30%, with only 5.0 mg.L^{-1}. It is important to stand out that the combination of these growth regulators was positive to the callus induction. Potentially, this morfogenetic event can occurs in all the plant tissue, preferentially in the one that has many meristematic cells (GRATTAPAGLIA; MACHADO, 1998). In agreement with these results, Arias and Huete (1983) reported that 2,4-D had an estimulatory effect in callus formation in peach palm. The effectiveness of auxins was described by Ferreira e Paqual (2008), who induced callus in stem fragments of *Ficus carica* L., and do not observed effect with citokinin supplementation. In opposition of this, callus were obtained in *Bixa orellana* L. shoot tips (CARVALHO et al., 2005), only utilizing BA in concentrations up to 2.22 µM and *Anthurium andraeanum* Lindl. by utilization of BA or 2,4-D (SANTOS, 2005).

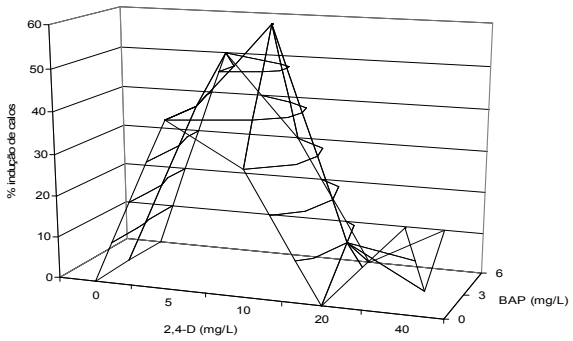

Figure 1. Response surface of callus induction in *B. gasipaes* shoot tips in relation to growth regulators concentrations (10.0 mg.L^{-1} 2,4-D: $Y=-3x^2+12x-6$ $R^2=1.00$; 3.0 mg.L^{-1} BAP: $Y=-1.2143x^2+6.9857x-5.4$

CONCLUSION

The disinfection of *Bactris gasipaes* shoot tips was efficient for 20 minutes of immersion in NaOCl 1.25%, which resulted in 90% of explants

without contamination and low oxidation. The greater callogenesis percentage was of 60%, reached at 10.0 mg.L^{-1} 2,4-D and 3.0 mg.L^{-1} BA combination.

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