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RESEARCH ARTICLE

EFFECT OF 2,4-D ON CALLUS INDUCTION IN LEAF EXPLANTS OF PEACH PALM (*BACTRIS GASIPAES* H.B.K.)

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ABSTRACT

The objective of this research was to determine the effect of dichlorophenoxyacetic acid (2,4-D) callus in peach friable calluses in leaf explants of *Bactris gasipaes* H.B.K., aiming for further induction of somatic embryos. The explants were inoculated in MS medium supplemented with 30.0 $g.L^{-1}$ sucrose, 8.0 $g.L^{-1}$ agar and 2,4-D (0.0, 0.31, 0.62, 1.25, 2.5, 5.0, 10.0, and 20.0 $mg.L^{-1}$). The cultures were kept in a growth room at 24±2°C under light conditions (50 $\mu mol.m^{-2}.s^{-1}$, photoperiod of 16 hours), and also under dark conditions, in factorial arrangement: 2 (light and dark conditions) x 8 (2,4-D concentrations), totaling 16 treatments. On the 14th day of cultivation, occurrences of oxidation and necrosis of the explants were observed and, on the 35th day, the formation of friable calluses, and the percentage of explant area covered by callus cells (EACC) were evaluated. Oxidation was more intense in the cultivations under light conditions, and reduced as the 2,4-D concentrationsincreased. However, the highest concentration, 20.0 $mg.L^{-1}$, caused necrosis of the explants. The concentration of 10 $mg.L^{-1}$ was the most efficient for friable callus induction reaching all the explants in the cultures kept in the dark. This concentration also resulted in the highest EACC, with an average of 56.5% of the explant area covered by callus cells.

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INTRODUCTION

*Bactrisgasipaes*H.B.K. (Arecaceae) is a tropical palm tree known as peach palm and native to South and Central America. It was domesticated by pre-Colombian people, who used its fruits, leaves and stipes in the region which is nowadays comprised between Bolivia and Honduras (Carvalho and Ishida, 2002; Miura, 1993). *B. gasipaes* have two important products -fruits and hearts of palm. The fruits have enhanced nutritional value especially due to the abundance of carotenes in the pulp and are widely used in the Amazonian cuisine, with great potential to reach other markets (Bovi, 2000; Ferreira, 2005). The high quality heart of palm of *B. gasipaes*has beentaking the place ofthose of *Euterpe edulis* Mart. in the West of Brazil and *E.oleracea*Mart. in the North (Carvalho and Ishida, 2002), the natural reserves ofwhich are already strongly reduced by predatory exploitation (Villachica, 1996).Additionally the heart of palm does not oxidize after harvesting, which makes the industrial process easier and cheaper than the other palms (Clement, 1987). *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 inefficient due to self-incompatibility, xenogamybeing the the predominant form of reproduction of this species. Other restrictive aspects for the reproduction by seeds include the long time taken to produce hybrid seeds and the limited cross pollination in the beginning of the flower induction, which results in the reduction of fruit production and formation of parthenocarpic fruits. The propagation also occurs by tillers, which can be found in a number of about five per plant. This form of propagation is very slow and often is not useful for breeding programs focused onfruit or heart of palmproduction. The limited number of available tillers prevents the formation of great populations genetically identical to the selected plant, which makes breeding programs based in this method inefficient (Almeida; Almeida, 2006; Souza *et al.,* 1996). In this context, methods of *in vitro* propagation have potential use in the clonal propagation of *B. gasipaes*. These methods 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 selected plants that havedesirable characteristics for breeding without the problems found in the traditional propagation methods (Rêgo *et al.,* 2009; Beltrão *et al.,* 2008;

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Grattapaglia and Machado, 1998). The propagation by tissue culture can be direct or indirect, the latter by callus formation and the return to the meristematic level from differentiated cells.The cultivation of calluses can result in shoot or somatic embryoinduction, following the transformation into new plants, which is a potential mass propagation. For thisthe exogenous supplement of growth regulators is needed (Silva *et al.,* 2010; Landa *et al.,* 2000). Conditions for callus formation and growth must be studied. Dichlorophenoxyacetic acid (2, 4-D) is the most used auxin in callogenesis and embryogenesis induction. This growth regulator stimulates cell division, controls cell elongation, and becomes the cells committed to the formation of embryos (Machakova *et al.,* 2008; Nogueira *et al.,* 2007).Thus, the objective of this research was to determine the most efficient concentration of 2,4-D for friable callus induction in leaf explants of *B. gasipaes*, aiming for further induction of somatic embryos.

MATERIALS AND METHODS

The experiments took place at the Plant Tissue Culture Laboratory of the Brazilian Agricultural Research Corporation (EmpresaBrasileira de PesquisaAgropecuária) – Rondônia Branch, in Porto Velho. Newly expanded green leaves were collected from tillers of peach palm plants kept at the experimental field. At the laboratory they were washed with distilled water and a detergent agent and, under aseptic conditions,immersed in 70%alcohol for one minute and 1.5%NaOCl with 0,1% Tween 20®for 20 minutes and rinsed three times in sterile distilled water. The leaves were cut into 1.0 cm^2 explants, which were individually inoculated (with the adaxial surface up) in test tubes containing MS (Murashige and Skoog, 1962) medium supplemented with 30.0 $g.L^{-1}$sucrose, 8.0 $g.L^{-1}$agar and 2,4-D (0.0, 0.31, 0.62,1.25, 2.5, 5.0, 10.0, and 20.0 $mg.L^{-1}$). The pH level of the medium was adjusted to 5.8±0,1before autoclaving at 120°C and 1 atm for 20 minutes. Half of the cultures were kept in a growth-chamber at 24±2°C under 16 hour photoperiod (50 $\mu mol.m^{-2}.s^{-1}$), and half of the cultures without light.The experimental design was entirely randomized, in factorial scheme: 8 (2,4-D concentrations) x 2 (light and darkness) totaling 16 treatments. Each treatment was composed ofthree replications of ten tubes. Fourteen days after inoculation occurrences of oxidation and necrosis were observed and at the 35th day the induction of friable calluses and the percentage of the explant area covered by callus cells (EACC) were evaluated by visual observation according to Cerqueira *et al.* (2002).

RESULTS AND DISCUSSION

Significant differences in the oxidation of the explants were observed in relation to their maintenance under light ordarkness, andalso in relation to the 2,4-D concentrations. The explants kept under light had a higher rate of oxidation compared to those kept in the darkness (Figure 1). The presence of light can promote the release of phenolic compounds, causing oxidation of both the explant and the culture medium (Nogueira *et al.,* 2007; Van Winkle *et al.,* 2003). Regarding the concentrations of 2,4-D, both in the absence and in the presence of light, the rate of oxidation decreasedas the concentrations of 2,4-D increased. The effect of 2,4-D in the decrease of oxidation was also reported by Sato *et al.* (2004) in explants of *Gallesiagorazema*Moq.

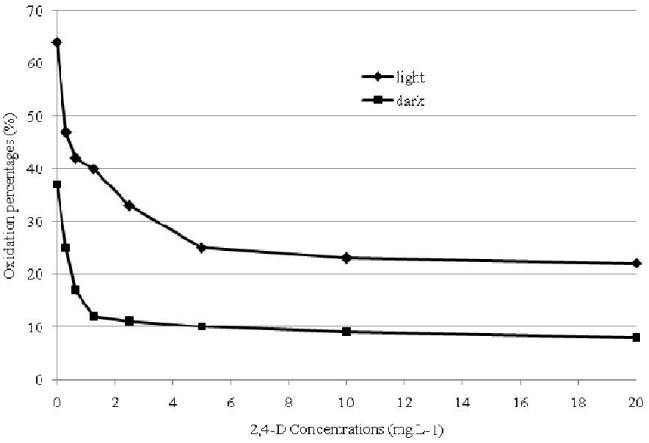

Figure 1. Oxidation in leaf explants of*B. gasipaes*in relation to different concentrations of 2,4-D in MS medium, 14 days after inoculation

At the concentration of 20 $mg.L^{-1}$2,4-D necrosis occurred in 29% of the explants kept in the darkness and in 35% of the explants under light.Necrosis can occur due to the injury caused by disinfectant agents like sodium hypochlorite, mainly in combination with surfactants as Tween 20®.However, the addition of surfactants in general is indicated because it overcomes the superficial tension and increases the contact of the disinfectant solution with the plant tissue (Hartmann *et al.,* 2011; Caldas *et al.,* 1998; Grattapaglia and Machado, 1998). On the 30th day of culture it was observed that the explants kept in the darkness had higher development of friable calluses than those kept under light. This fact was mainly due to the incidence of oxidation and necrosis under light conditions. According to Machakova *et al.* (2008), primary cellular divisions that lead to callus formation can be inhibited by light conditions. Moreover, light promotes the release of phenolic compounds, the oxidation of which can limit the action of the auxin. This was observed by Nogueira *et al.* (2007), who evaluated the effect of different concentrations of 2,4-D on callus induction in leaf explants of*Byrsonima intermedia* A. Juss.and observed that the presence of light favored the production of phenolic compounds and these substancescould have affected the activity of the 2,4-D growth regulator. Bassan *et al.* (2006), studying oxidation of nodal segments and apices of *Peltophorumdubium* (Spreng.) Taub., did not observe oxidation, andconcluded that the formation of phenolic compounds in explants can be dependent on the peculiarities of the species or on the age of the explants. In the absence of 2,4-Dno callus induction was observed. In general, auxins are necessary for callus initiation and growth, as they are responsible for the first cellular divisions on the explant and control the processes involving cellular growth and elongation (TAIZ and ZEIGER, 2010). The need for exogenous growth regulators for callus induction in leaf explants of *B. gasipaes* was previously reported by Santos *et al.* (2012). Regarding the effect of 2,4-D onthe treatments kept in the darkness, callus induction enhanced as the concentration of 2,4-D increased. Callus induction was observed in 12% of the explants at the concentration of 2.5 $mg.L^{-1}$, 52% at 5.0 $mg.L^{-1}$, and 100% at

10.0 mg.L^{-1} 2,4-D (Figure 2),decreasing to 8% at 20 mg.L^{-1}. This decrease can be explained by the toxic effect that high concentrations of auxins have on leaf tissues of certain species, causing the inhibition of the formation of friable calluses (Grattapaglia and Machado, 1998). Lower concentrations (0.31, 0.62, and 1.25 mg.L^{-1}) were not efficient for callus induction.In the treatments under light, callus induction only occurred on the concentration of 10 mg.L^{-1}, where it reached 50% of the explants.

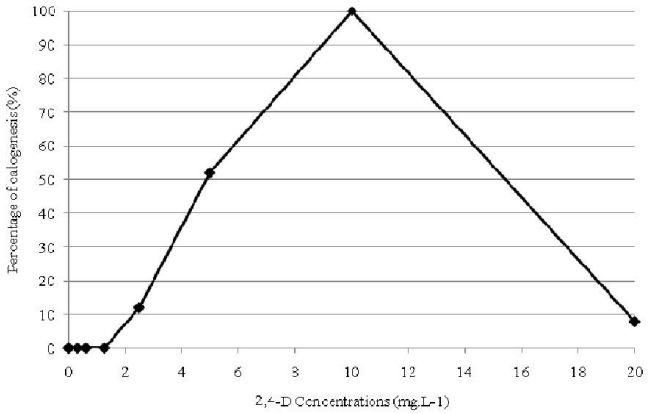

Figure 2. Induction of friable calluses in leaf explants of *B. gasipaes*in relation todifferent concentrations of 2,4-D in MS medium,under dark conditions, 35 days after inoculation

Navroski *et al.* (2012) attribute the formation of friable calluses, supplementing the medium only with auxins, to the presence of endogenous cytokinins in the explants, resulting in a hormonal balance adequate for this morphological event.Santos *et al.* (2012) also studied callus induction in leaf explants of *B. gasipaes* and observed 70% of callus induction with 10.0 mg.L^{-1} 2,4-D in the darkness. Santos *et al.* (2010b) reported 60% of callus induction on shoot apexof *B. gasipaes* plants in the darkness using 10 mg.L^{-1} 2,4-D in combination with 3.0 mg.L^{-1} BA. Also concerning callus induction on shoot apex of *B. gasipaes*, Arias andHuete (1983) had the best results using concentrations of 2,4-D from 20 to 50 mg.L^{-1}. Werner studied callus induction in explants from juvenile leaves of *Caesalpiniaechinata* Lam. using concentrations from 5.0 to 20.0 mg.L^{-1} 2,4-Dand reported calluses in approximately 45 and 55% of the explants, under dark and light conditions respectively. Santos *et al.* (2003) tested callus induction in *Coffeaarabica*L. leaf explants, reporting percentages of 61, 67, and 69% at the concentrations of 0.5, 1.0, and 1.5 mg.L^{-1} respectively. In Figure 3 it is possible to note that the explant area covered by callus cells (EACC) of the explants kept in the darkness followed a similar pattern observed in the callus induction percentage (Figure 2), also in the darkness.The EACC increased as the 2,4-D concentrations raised up to 10 mg.L^{-1}, and then decreased. This concentration resulted in an average of 56.5% of the explant area covered by callus cells, which was highly different from the EACC obtained with the other concentrations. The concentrations of 2.5, 5.0, and 20.0 mg.L^{-1} resulted in 2.5, 19.5 and 2.0% EACC respectively. In the treatments under light, callus induction only occurred in the 2,4-D concentration of 10 mg.L^{-1} and reached an average of 19.5% EACC. Santos *et al.* (2010c) induced callus in ribs of not-expanded leaves of *B. gasipaes* and observed that the highest EACC was obtained in MS medium supplemented with 1.10 mg.L^{-1} 2,4-D in combination with3.66 mg.L^{-1} 2iP.

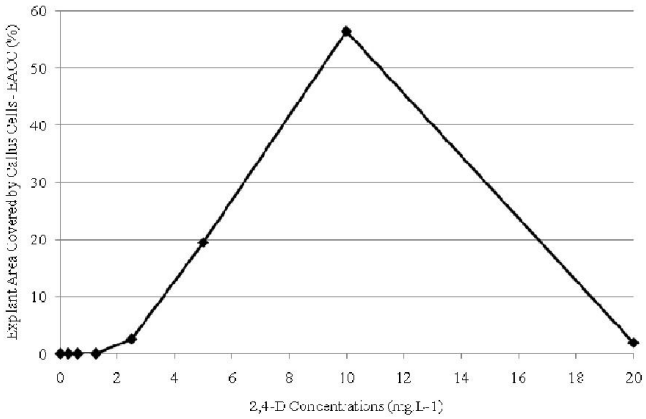

Figure 3. Average percentages of explant area covered by callus cells (EACC) in leaf explants of *B. gasipaes*in relation to different concentrations of 2,4-D in MS medium,under dark conditions, 35 days after inoculation

Conclusion

Friable calluses of *B. gasipaes* can be achieved by inoculating leaf explants in MS medium supplemented with 10 mg.L^{-1} 2,4-D, resulting in callogenesis in all the explants kept in the dark and an average of 56.5% of the explant area covered by callus cells.

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