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Short Communication

Organogenesis in zygotic embryos and hypocotyls of physic nut (*Jatropha curcas* L.) under 6-benzylaminopurine levels

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Abstract
Physic nut had a great potential for biodiesel production and presents higher oil content. The aim of this study was obtain organogenesis in zygotic embryos and hypocotyls of physic nut using 6-benzylaminopurine (BAP). Seeds were disinfected and after then cotyledons were removed; they were in vitro germinated at 0, 1, 2, 3, 4, 5 and 6 mg.L^{-1} BAP. Hypocotyls were excised from germinated seeds with 45 days old and cultured at 0, 1, 2.5 and 5 mg.L^{-1} BAP. Callus from zygotic embryos were cultured at double-phase medium being liquid medium supplemented with 1, 2.5 and 5 mg.L^{-1} BAP. The largest percentage of callus formation in zygotic embryos occurred at 6 mg.L^{-1} BAP. On the other hand, to induce shoot from zygotic embryos the best BAP level was 4 mg.L^{-1}. Callogenesis in hypocotyls can be efficiently induced with 1 mg.L^{-1} BAP. Double-phase medium results in lowest protuberances rate, varying from 0 to 3 per callus. In order to induce callus from zygotic embryos and hypocotyls of physic nut, BAP is sustainable.
Key words: Callogenesis; BAP; Tissue culture; *In vitro* morphogenesis.

Introduction

Jatropha curcas L. (Euphorbiaceae) is found in tropical and subtropical regions. This species had a great potential for biodiesel production and presents high oil content (Sujatha et al. 2008). Moreover, also had medicinal applications, such as antiseptic, purgative and cicatrizant (Faria et al. 2006).

These species spread by seed and vegetatively, nevertheless, technologies for seedling production are insufficient (Costa et al. 2011). On the other hand, tissue culture studies are yet restricted for these species, but it is very important to overcome some difficult presented for physic nut, such as culture irregular originated from seeds.

The clonal propagation by tissue culture can produce uniform cultures (Costa et al. 2010), and the *in vitro* regeneration is indispensable to the genetic transformation step, required to remove to the toxicity present in physic nut. However basic information about *in vitro* behaviour of this plant is necessary to establish efficient protocols that serve as a prelude for genetic breeding through biotechnological tools.

The aim of this paper was to obtain organogenesis in zygotic embryos and hypocotyls of physic nut using BAP.

Materials and methods

Disinfection and in vitro germination of seeds

Seeds were disinfected through immersion in ethanol (70%) during 1 min followed by immersion in commercial bleach (2.5% NaOCl) for 10 min and rinsed three times in sterilized distilled water. Cotyledons were removed from seeds after disinfection. Seeds were germinated on MS basal medium (Murashige and Skoog 1962) supplemented with 30 g.L^{-1} sucrose and solidified with 6 g.L^{-1} agar. The pH of all media was adjusted to 5.8.

Effects of BAP on zygotic embryos without cotyledons

Seeds disinfected were *in vitro* germinated on basal medium with the treatments: 0, 1, 2, 3, 4, 5 or 6 mg.L^{-1} BAP. The rooting (%), callus (%) and shoot (%) were evaluated after 60 days of *in vitro* culture.

Effects of BAP in hypocotyls

Hypocotyls from zygotic embryos without cotyledons cultured at different BAP levels (explant with 45 d) were cultured on the basal medium supplemented with the treatments: 0, 1, 2.5 or 5 mg.L^{-1} BAP. The rooting (%), callus in any region of hypocotyls (%) and complete callus (%) were evaluated after 20 d of *in vitro* culture.

Effects of double-phase medium with BAP on callus

Callus from zygotic embryos without cotyledons (explant with 45 d) cultured at 4 or 6 mg.L^{-1} BAP were cultivated on double-phase medium, 15 mL solid basal medium and 1 mL liquid basal medium supplemented with the treatments: 1, 2.5 or 5 mg.L^{-1} BAP. The protuberance number, callus with protuberances (%), oxidization (%) and survival (%) were evaluated after 24 d of *in vitro* culture.

Culture conditions and statistical analysis

All the experiments were kept in a growth room with temperature of 25ºC ± 2ºC and 16 h of photoperiod under a light intensity of 62 µM.m^{-2}.s^{-1} obtained with white fluorescent lamps. The experimental design was a complete randomized with five replicates of five explants. The data was submitted in a normality analysis for the Lilliefors´s test and, analysis of variance followed by regression analysis or Duncan´s test at the level of 5% of error. All analysis were done following the procedures of the software GENES (Cruz 2001). Variables from counting were transformed to $\sqrt{x+0.5}$ and variables from percentage were transformed to arcsin $\sqrt{x/100}$.

Results and discussion

Callus formation in zygotic embryos without cotyledons was promoted using BAP, these results followed a positive linear regression, the largest percentage of callus formation occurred at 6 mg.L^{-1} BAP, it suggests that higher BAP levels can increase callus rate (Fig. 1A and 2A). Similar results were found in decoated mature seed of *Jatropha panduraefolia* which occurred proliferation of endosperm producing profusely growing callus with differentiated tracheidal cells, however induced through 2,4-D (2,4-

dichlorophenoxyacetic acid), Kin (6-furfurilamonopurine) and yeast extract (Srivastava 1971).

In order to induce shoot from zygotic embryos the maximum efficacy of BAP level was 4 mg.L^{-1}, these results followed a positive quadratic curve (Fig. 1B and 2B). However, shoot number was close to 1-2. Similar results was found in *Jatropha panduraefolia* which endosperm callus sub-cultured occurred differentiation of shoots induced through Kin (6-furfurilamonopurine) (Srivastava and Johri 1974).

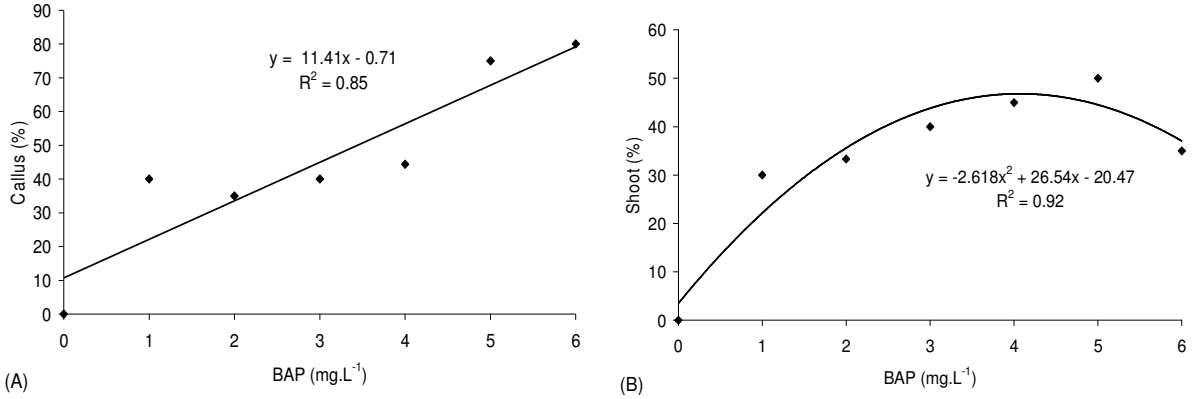

Figure 1. Characteristics observed in zygotic embryos without cotyledons of physic nut (*Jatropha curcas* L.) after 60 days of *in vitro* culture. Fig. 1A, Percentage of callus and Fig. 1B, Percentage of shoots.

BAP decreases rooting percentage in zygotic embryos, these results followed a negative linear regression (data not shown). BAP inhibits rooting in hypocotyls explants. Hypocotyls produce rooting in absence BAP only, approximately 16% (data not shown). Similar results were found in squash (*Cucurbita pepo* L.) because increases in BAP level decreases rooting percentage in cotyledons (Silva et al. 2006).

Hypocotyls formed higher callus rate, 100% of callus in any region of hypocotyls and approximated 25% of complete callus, both results from these variables followed a negative linear regression (Fig. 3A and 3B). In hypocotyls, BAP induces callus only, just epicotyls developed (data not shown). BAP produces higher shoot rate normally, but for zygotic embryos without cotyledons and hypocotyls of physic nut, the BAP had favoured higher percentage of callus induction. Similar results was found in *Arachis hipogaea*, which BAP promotes only callus, on the contrary, these authors waited shoot production (Furtado et al. 2007). The BAP was efficient to produce callus in grapevine cv. Merlot too (Carvalho et al. 2011).

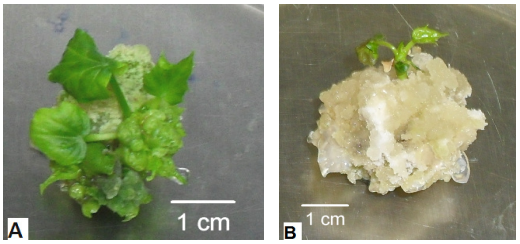

Figure 2. Callus of physic nut (*Jatropha curcas* L.). (A) Complete callus from zygotic embryos at 6 mg.L^{-1} BAP. (B) Callus and shoot originated from zygotic embryos at 1 mg.L^{-1} BAP. Bar = 1 cm".

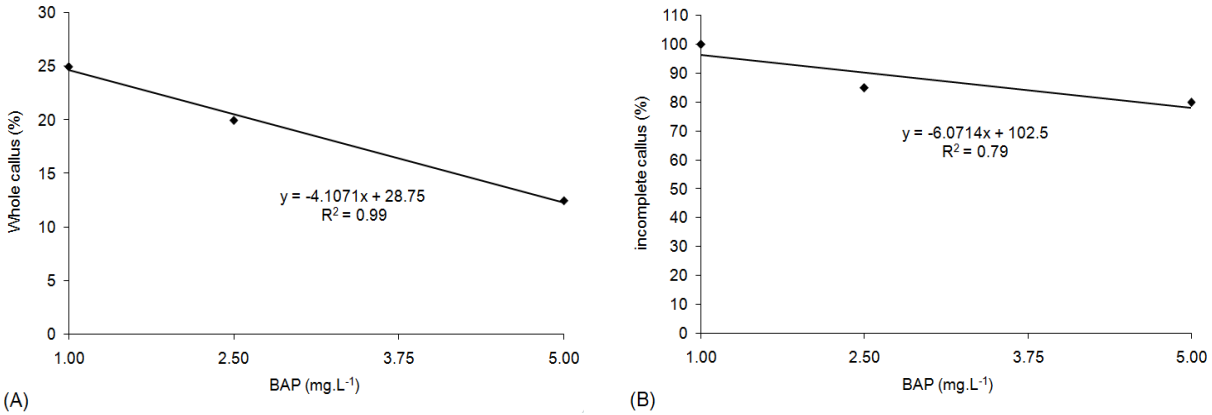

Figure 3. Characteristics observed in hypocotyls of physic nut (*Jatropha curcas* L.) after 20 days of *in vitro* culture. (A) Percentage of complete callus. (B) Percentage of explants with callus in any region of hypocotyls.

The origin of callus (i.e., prior culture medium) influence the result obtained in the induction of callus protuberances during double-phase medium culture, callus originated from culture medium with 6 mg.L^{-1} BAP showed better results than those originated from medium supplemented with 4 mg.L^{-1} BAP (Table 1). Double-phase medium results in lowest protuberances rate, varying from 0 to 3 per callus, the best result was found at 5 mg.L^{-1} BAP, which 70% of callus formed protuberances, and this level promotes lowest percentage of oxidization in this callus (Table 1). The tissue oxidization normally occurs in woody plants tissues, but physic nut tissues just presented oxidization in this experiment, this fact can be due to type of media used, such as a liquid medium layer involved the explants. However other reasons can be involved.

The quantity of liquid medium (1 mL) was little, because in some test tubes had no more medium at the second day. This fact can be related to lowest protuberance

number. It suggests testing largest quantity of liquid medium for double-phase culture.

In order to induce callus from zygotic embryos and hypocotyls of physic nut, BAP is sustainable at 6 mg.L^{-1} and 1 mg.L^{-1}, respectively.

Table 1. Characteristics observed in callus of physic nut (*Jatropha curcas* L.) after 24 days of *in vitro* culture on double-phase medium on protuberances number (PN), percentage of callus with protuberances (CP), percentage of callus with oxidization (O) and percentage of callus survival (S).

BAP (mg.L^{-1})	PN	CP (%)	O (%)	S (%)
Callus from culture with 4 mg.L^{-1} BAP				
1.0	0.0^{c}	0.0^{d}	65.0^{b}	65.0^{c}
2.5	0.0^{c}	0.0^{d}	100.0^{a}	75.0^{bc}
5.0	1.0^{b}	35.0^{c}	85.0^{ab}	85.0^{a}
Callus from culture with 6 mg.L^{-1} BAP				
1.0	1.4^{b}	55.0^{b}	90.0^{a}	90.0^{a}
2.5	3.0^{a}	65.0^{ab}	100.0^{a}	90.0^{a}
5.0	3.0^{a}	70.0^{a}	30.0^{c}	90.0^{a}

Means within a column for each parameter followed by the same letter are not different at $P<0.05$ by Duncan´s test.

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