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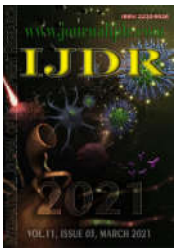

RESEARCH ARTICLE OPEN ACCESS

ORGANOGENESIS INDUCTION IN FOLIAR EXPLANTS AND NODAL SEGMENTS OF *SOLANUM PANICULATUM* L

Faustino S. Luciely[1], Silva R. Rodrigo Kelson[2], da Silva M. Thiago[1], Vieira J. Maílson[2], Siqueira M. Elton Aparecido[2] and Pereira N. Geisianny[1]

[1]Department of Biological and Environmental Sciences, Federal University of Grande Dourados
[2]Department of Agrarian Sciences, Federal University of Grande Dourados

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Corresponding author:
Thiago da Silva Messias

ABSTRACT

Solanum paniculatum L. popularly known as jurubeba, is a shrub that has pharmacological importance. In this way, micropropagation emerges as an alternative to enable the production of seedlings on a large scale, presenting several advantages from the multiplication of genetic material, for the exchange or evaluation of germplasms, to the production of virus free seedlings. The aim of this study was to perform an efficient in vitro establishment of organogenesis from leaf explants and nodal segments of jurubeba under the effect of different concentrations of 6-benzylaminopurine (BAP) and naphthalene-acetic acid (NAA), (BAP: 0.0, 1.0, 2.0, 4.0 or 8.0 mg L^{-1}; NAA: (0.0, 0.5 or 1.0 mg L^{-1}). The experiment was carried out in DIC (Entirely Randomized Design), and elaborated in a 3x5 factorial scheme, showing statistically significant differences ($p < 0.05$) for all variables analyzed. To induce greater callus formation in leaf explants and nodal segments, the use of BAP and NAA (1.0 mg L^{-1}) is recommended. For bud formation, from leaf explants, it is recommended to promote the use of 1.0 mg L^{-1} NAA without using BAP. For nodal segments, a greater number of shoots is obtained with 8.0 mg L^{-1} of BAP without using the NAA growth regulator.

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INTRODUCTION

The exploitation of natural resources is becoming a growing concern worldwide, especially in the Cerrado, as the second largest biome in Brazil, it has a great native diversity of herbaceous, shrub and tree fruit species. In this regard, there is a scenario in which the loss of native flora has been occurring, mainly, related to disorderly occupation for urban expansion, extraction of raw material, agropastoral and burning activity, which already reaches more than 50% of the biome (MMA, 2018). The genus Solanum, composed of about 1500 species, is the most representative of the Solanaceae family, constituting one of the largest groups among angiosperm and dicotyledonous plants (Gomes *et al*., 2014). *Solanum paniculatum* L. popularly known as jurubeba, jurubeba-real or even jurubebinha is a shrub that can reach up to two meters in height, being generally found in the Brazilian Midwest, especially in the Cerrado (MS, 2015). This plant is resistant to drought, and can be used for several purposes, In the fruits are found active compounds and secondary metabolites, such as steroids, saponins, alkaloids and glycosides, of pharmacological importance, for specific use against anemia and liver problems. In addition to fruits, the leaves and roots of jurubeba have been widely used to stimulate digestive functions and reduce swelling of the liver and gallbladder, in addition to being useful against chronic gastritis, intermittent fevers and the prevention of uterine tumors (Miranda *et al*., 2013). For culinary purposes, the fruit is used as a spice, in preserves and additives in sugarcane in several regions of Brazil (Lorenzi *et al*., 2002). Fruit species have high economic and social value and are thus commercially exploited. Thus, micropropagation emerges as an alternative to enable the production of seedlings on a large scale (Saptari *et al*., 2017), which begins with the extraction of small fragments of tissue from different parts of the plant, called explants, being these are transferred in aseptic conditions to an adequate culture medium, so that periodic subcultures allow the amplification of plant material until obtaining an entire plant (Lema-rumińska *et al*., 2014).

In vitro cultivation techniques have several advantages, from the multiplication of genetic material, for the exchange or evaluation of germplasms, to the production of virus-free seedlings, in addition to the possibility of propagation at any time due to controlled laboratory conditions. Being a tool with high potential in plant breeding (Vijendra *et al*., 2017). Plants obtained by asexual or vegetative propagation are equal to each other and equal to the parent plant. In micropropagation, *in vitro* regeneration can occur by organogenesis through the direct pathway, where a plant organ is induced and directly develops an explant, without going through the initial callus phase or indirect pathway, where there is proliferation and growth of callus and later development sprouting (Faleiro *et al*., 2011). The vast majority of plant species require exogenous supplementation of growth regulators that are synthetic substances, which stand out in five main classes, being cytokinins, auxins, gibberellins, abscisic acid and ethylene for their *in vitro* development (Pinhal *et al*., 2011).The purpose of this study was to perform an efficient *in vitro* establishment of organogenesis from leaf explants and nodal segments of jurubeba under the effect of different concentrations of 6-benzylaminopurine (BAP) and naphthalene-acetic acid (NAA).

MATERIAL AND METHODS

Location of the experiment and material: The experiment was conducted at the Plant Biotechnology Laboratory of the Center for Biotechnology and Genetic Improvement of Sugarcane at the Federal University of Grande Dourados – UFGD / Dourados – MS. First, jurubeba seeds were collected on the campus of the Federal University of Grande Dourados to obtain seedlings germinated *in vitro*. In a horizontal laminar flow chamber, the seeds were disinfected following the protocol: 2 minutes in 70% alcohol (v/v), 5 minutes in 2.5% sodium hypochlorite (commercial) followed by 3 washes with autoclaved distilled water. After disinfestation, the seeds were inoculated in 250 mL flasks containing 50 mL of MS medium (Murashige & Skoog, 1962) supplemented with 30 g L^{-1} of sucrose, solidified with 6 g L^{-1} of agar, in each bottle inoculated 5 seeds are kept keeping the greatest distance between them.

Inoculation of explants and concentrations of growth regulators: After 45 days of seedling germination, they were used as a source of explants for the *in vitro* organogenesis experiment. The culture medium used in the organogenesis experiment was the MS medium consisting of combinations of macro and micronutrients essential for plants, supplemented with 30 g L^{-1} of sucrose and defined with different concentrations of growth regulators (6-benzylaminopurine - BAP: 0.0, 1.0, 2.0, 4.0 or 8.0 mg L^{-1}) and (naphthalene-acetic acid - NAA: 0.0, 0.5 or 1.0 mg L^{-1}). The pH of the medium was adjusted to 5.8 and then solidified with 6 g L^{-1} of agar. After preparing the culture medium, it was autoclaved at 121 °C and pressure of 1.05 kg cm^{-2} for 20 minutes. In a horizontal laminar flow chamber under aseptic conditions with the aid of glassware necessary for the *in vitro* cultivation technique, the seedlings were removed from the flasks, one by one, and in Petri dishes, leaf explants of approximately 1 cm^2 were extracted and explants of nodal segments with a lateral yolk. After the excision of the plant material, the explants were inoculated in test tubes containing 15 mL of MS medium, and the leaf explants were inoculated with the abaxial portion in contact with the culture medium, whereas the nodal segments were inoculated vertically (mini cuttings).

The experiment was carried out in DIC (Completely Randomized Design), and elaborated in a 3x5 factorial scheme, (3 concentrations ofNAA x 5 BAP) totaling 15 treatments, each treatment consisting of 5 repetitions, which consisted of 5 test tubes each, containing one explant per tube, thus resulting in 25 experimental plots. After the explants were inoculated, the test tubes were transferred and kept in a growth room at 25 ± 2 °C for 7 days in the absence of light and, subsequently, maintained in a 16-hour photoperiod (45 μmol m^{-2} s^{-1}) by white fluorescent lamps. (D.A.I.) 30 days after inoculation of the *in vitro* organogenesis experiment, the callus intensity, the number of shoots and the fresh and dry mass of the calluses and jurubeba shoots were evaluated. For callus intensity, a score was assigned to each callus, on a scale of 0 to 3, where 0 corresponded to the absence of the callus and 3 to the largest callus formation.

Statistical treatment: The data obtained were transformed into $\sqrt{5+0.5}$, after the transformation they were subjected to analysis of variance (ANOVA) and, when significant, the means were compared by the Tukey Test, at the level of 5% probability of error. Data analysis was performed using the GENES software (Cruz, 2016).

RESULTS

It was observed that there were statistically significant differences for all variables analyzed at the level of 5% probability of error ($p < 0.05$), demonstrating the interaction between growth regulators and the concentrations used. The data obtained with leaf explants (Table 1) show that, for callus intensity, the NAA concentrations of 0.5 and 1.0 mg L^{-1} combined with 1.0 mg L^{-1} of BAP do not differ statistically between themselves, however, they have higher means (2.54 and 2.60) in relation to the treatment without NAA. In this case, the use of 0.5 mg L^{-1} of the NAA growth regulator is advantageous, in relation to cost, in addition to providing satisfactory results for callus induction in jurubeba leaf explants. For all averages obtained using 4.0 and 8.0 mg L^{-1} of BAP, there were no statistically significant differences when compared to each other, except for the interactions of 0.0 and 2.0 mg L^{-1} of BAP with absence and 1.0 mg L^{-1} of NAA, these treatments have lower mean callus intensity.

For the number of shoots, the highest average was 2.80 in the absence of BAP with 1.0 mg L^{-1} of NAA, and the lowest averages (0.50) were obtained in treatments with 0.5 mg L^{-1} NAA with BAP interaction (0.0, 1.0, 2.0 4.0 or 8.0 mg L^{-1}). Due to the transformation of the data for statistical analysis, the means 0.50 correspond to zero, as well as for the other variables analyzed, thus, regardless of the concentration of BAP combined with 0.5 mg L^{-1} NAA does not favor the induction of shoots in leaf explants of the species under study, as well as for 2.0 mg L^{-1} of BAP. However, it is recommended to use only 1.0 mg L^{-1} of NAA in the absence of BAP to obtain a greater number of shoots. For the fresh weight variable, the highest average corresponds to 2.22 using the combination of 1.0 mg $L^{-1}$of both growth regulators, however, it does not differ statistically from 2.19 with 0.5 mg L^{-1} NAA with an interaction of 1.0 mg L^{-1} BAP, which are recommended for obtaining biomass of jurubeba, in addition to the use of low NAA concentration. For dry mass, the situation is similar to the callus intensity results, therefore, it is observed that the NAA concentrations of 0.5 and 1.0 mg L^{-1} combined with 1.0 mg L^{-1} of BAP do not differ statistically from each other, presenting higher averages (0.86 respectively) and in relation to the treatment with absence of NAA (0.81), to obtain a greater weight of dry mass, only BAP 1.0 mg L^{-1} can be used.

When using nodal segments of jurubeba (Table 2), the data show that the highest mean (2.05) of callus intensity was found using 1.0 mg L^{-1} of the phytoregulators BAP and NAA, while the lowest mean was obtained with the absence of NAA and 1.0 mg L^{-1} of BAP. This indicates that, for greater callus induction, the interaction between NAA and BAP in the proportion of 1.0 mg L^{-1} is necessary. Much of the averages obtained in the number of shoots found in the nodal segments was similar, between the concentrations of growth regulators used, with no statistically significant differences, except for treatments with 8.0 mg L^{-1} of BAP with interaction of 0.5 and 1.0 mg L^{-1} of NAA. These have the lowest mean values (1.36 and 1.14, respectively). To obtain shoots in nodal segments, the use of synthetic hormones is not necessary, since the average 1.60 does not differ statistically from the highest average for the variable number of shoots (2.11). The interaction of 1.0 mg L^{-1} of BAP and NAA provided the highest weight of fresh and dry mass, without great variation in the weight of the biomass obtained with the increase in the concentration of BAP, which shows that for this species, the concentration ideal for higher biomass yield is 1.0 mg L^{-1} of BAP with a minimum concentration of NAA (1.0 mg L^{-1}).

Table 1. Effect of interactions between 6-benzylaminopurine (BAP) and naphthalene-acetic acid (NAA) on callus intensity, number of sprouts and fresh and dry mass, in leaf explants of *Solanum paniculatum* L. after 30 days of *in vitro* culture

Explant	Variable	BAP (mg L^{-1})	NAA (mg L^{-1})		
			0.0	0.5	1.0
Sheets	Callus intensity	0.0	0.50 Bc	2.06 Ab	2.00 Ab
		1.0	2.04 Ba	2.54 Aa	2.60 Aa
		2.0	1.38 Bb	2.29 Aab	2.47 Aa
		4.0	2.31 Aa	2.23 Aab	2.26 Aab
		8.0	2.21 Aa	2.35 Aab	2.35 Aab
	Number of shoots	0.0	0.50 Bb	0.50 Ba	2.80 Aa
		1.0	2.35 Aa	0.50 Ba	2.14 Ab
		2.0	0.50 Ab	0.50 Aa	0.50 Ac
		4.0	2.52 Aa	0.50 Ba	0.50 Bc
		8.0	2.41 Aa	0.50 Ba	0.50 Bc
	Fresh mass (g)	0.0	0.50 Bb	1.78 Ab	1.91 Ab
		1.0	1.84 Ba	2.19 Aa	2.22 Aa
		2.0	1.97 Aa	2.12 Aa	2.04 Aab
		4.0	1.97 Aa	2.05 Aab	2.05 Aab
		8.0	1.93 Aa	2.12 Aa	2.13 Aab
	Dry mass (g)	0.0	0.50 Bb	0.76 Ab	0.79 Ab
		1.0	0.81 Aa	0.86 Aa	0.86 Aa
		2.0	0.81 Aa	0.84 Aa	0.83 Aab
		4.0	0.80 Aa	0.82 Aab	0.82 Aab
		8.0	0.80 Aa	0.84 Aa	0.84 Aab

Equal capital letters on the lines do not differ statistically. Same lowercase letters in the column do not differ statistically. The differences found are at the level of 5% probability of error ($p<0.05$), by the Tukey Test.

Table 2. Interaction between 6-benzylaminopurine (BAP) and naphthalene-aceticacid (NAA) in callus intensity, number of sprouts and freshand dry mass, in nodal segments of *Solanum paniculatum* L. after 30 days of *in vitro* culture.

Explant	Variable	BAP (mg L^{-1})	NAA (mg L^{-1})		
			0.0	0.5	1.0
Nodal Segments	Callus intensity	0.0	1.65 Aa	1.76 Aa	1.66 Ab
		1.0	1.62 Ba	1.72 Ba	2.05 Aa
		2.0	1.89 Aa	1.93 Aa	1.95 Aab
		4.0	1.75 Aa	1.97 Aa	2.01 Aab
		8.0	1.83 Aa	1.84 Aa	1.89 Aab
	Number of shoots	0.0	1.60 Aa	1.98 Aa	1.50 Aa
		1.0	1.69 Aa	1.68 Aab	1.56 Aa
		2.0	1.68 Aa	1.67 Aab	1.34 Aa
		4.0	1.86 Aa	1.40 Aab	1.50 Aa
		8.0	2.11 Aa	1.36 Bb	1.14 Ba
	Fresh mass (g)	0.0	1.64 ABa	1.61 Bb	1.86 Ab
		1.0	1.50 Ca	1.84 Bab	2.19 Aa
		2.0	1.70 Ba	2.00 Aa	2.18 Aa
		4.0	1.63 Ba	2.09 Aa	2.08 Aab
		8.0	1.46 Ba	2.04 Aa	2.06 Aab
	Dry mass (g)	0.0	0.74 Aa	0.73 Ab	0.78 Ab
		1.0	0.71 Ca	0.78 Bab	0.86 Aa
		2.0	0.75 Ba	0.82 Aa	0.85 Aa
		4.0	0.74 Ba	0.83 Aa	0.83 Aab
		8.0	0.70 Ba	0.82 Aa	0.83 Aab

Equal capital letters on the lines do not differ statistically. Same lowercase letters in the column do not differ statistically. The differences found are at the level of 5% probability of error ($p<0.05$), by the Tukey Test.

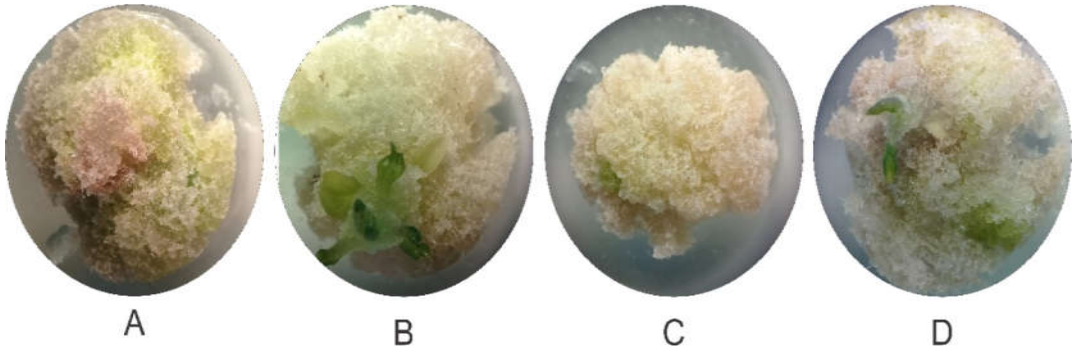

Figure 1. *In vitro* organogenesis indirectly in leaf explants and nodal segments. Callus intensity aspect in leaf explants (A) and nodal segments (C) of *Solanum paniculatum* L. Images B and D represent the formation of buds in leaf explants and nodal segments, respectively. Images were obtained 30 days after inoculation

The (Figure 1) illustrates the callus formation in both explants evaluated, in which image A represents the callus intensity from leaf explants and image C refers to the callus intensity in nodal segments. In image B and D, they refer to leaf explants and nodal segments respectively, where the initial phase of proliferation and callus growth is observed and subsequently the appearance of sprouts in both images. According to the figure, it can be said that the experiment carried out was of *in vitro* organogenesis indirectly, that is, passing through the initial callus phase.

DISCUSSION

From the data obtained in the present work, it can be observed that the interaction between cytokinin and auxin has effects on the formation of corns, both in leaf explants and in nodal segments of jurubeba. The composition, as well as the concentration of the growth regulator used, are determining factors for the development of tissue culture systems. George *et al*. (2008) argue that auxins may be unnecessary and may even reduce the germination capacity in some species, since the increase in cytokinin may increase the formation of sprouts, as well as their quality. The data obtained are in agreement with these authors, where it is observed that the highest number of shoots was obtained with 8.0 mg L^{-1} of BAP and with the absence of NAA. In this experiment, two types of explants (leaf and nodal segments) were tested. Carvalho *et al*. (2011), reports that the formation of calluses in some species is influenced by the types of explants used and that the internodal segments are more successive for the formation of calluses compared to leaf explants. In this work the results are similar, however, the nodal segments stand out as excellent sources of explants for the formation of corns in comparison to the leaf explants of jurubeba. In this work the results are similar, however, the nodal segments stand out as excellent sources of explants for the formation of corns in comparison to the leaf explants of jurubeba.

The highest average callus intensity was obtained using 1.0 mg L^{-1} of NAA and BAP, indicating that the interaction of these growth regulators is necessary to form greater plant biomass. Jaskani *et al*. (2008) using 1 μM NAA in the culture medium for *Vitis vinifera* L. cv. Perlette obtained 40% callus formation in nodal segments and 80% callus in leaf explants, in addition to inducing rooting in these explants. The greater callus intensity obtained by Rodrigues and Almeida (2010) occurred when BAP was used, which is in accordance with the data presented. As Cerqueira *et al*. (2002), when performing the callus induction in *Tridax procumbens* Linn, 100% of the explants area was covered with calluses when 2.0 mg L^{-1} of NAA + 2.0 mg L^{-1} of BAP was used. In the two explants used, the highest averages of fresh and dry mass were obtained with the interaction of BAP and NAA at 1.0 mg L^{-1}. For Cerqueira *et al*. (2002), the best results in fresh callus mass occurred when BAP and NAA were used in the concentration of2.0 mgL^{-1} and obtained greater dry mass when interacting with cytokinin, demonstrating that the interaction between these growth regulators really influences development of plant cells. These data are in agreement with the data presented in this work, where the interaction BAP and NAA provided greater weight of fresh and dry mass.

CONCLUSION

In the present study, it was observed that to induce greater callus formation in leaf explants and nodal segments, the use of BAP and NAA in the concentration of 1.0 mg L^{-1} is recommended. For bud formation, from leaf explants, it is recommended to promote the use of 1.0 mg L^{-1} of NAA in the absence of BAP. For nodal segments of jurubeba, a greater number of shoots is obtained with 8.0 mg L^{-1} of BAP without the use of the NAA growth regulator, however, the absence of both phytoregulators is recommended to obtain satisfactory results, in the induction of shoots of jurubeba.

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