Notas Científicas

Solidifying agents and activated charcoal for in vitro culture of *Solanum sessiliflorum*

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Abstract – The objective of this work was to evaluate the effects of the solidifying agents agar and phytagel and of activated charcoal on the in vitro cultivation of two maná cubiu (*Solanum sessiliflorum*) varieties: Thaís and Santa Luzia. The phytotechnical characteristics analyzed included number of leaves, number of roots, shoot and root length, and fresh matter of shoot and root. Regardless of the variety, phytagel was superior to agar as a culture medium. A greater number of leaves and longer shoots were observed in the Santa Luzia variety, in the absence of charcoal. The Thaís variety showed longer shoots and roots in the presence of charcoal.

Index terms: maná cubiu, micropropagation, plant tissue culture, phytagel, Solanaceae.

Agentes solidificantes e carvão ativado no cultivo in vitro de *Solanum sessiliflorum*

Resumo – O objetivo deste trabalho foi avaliar os efeitos dos agentes solidificantes ágar e phytagel e do carvão ativado no cultivo in vitro de duas variedades de maná cubiu (*Solanum sessiliflorum*): Thaís e Santa Luzia. As características fitotécnicas analisadas incluíram número de folhas, número de raízes, comprimento de parte aérea e raiz, e massa de matéria fresca de parte aérea e raiz. Independentemente da variedade, o phytagel foi superior ao ágar como meio de cultura. Observou-se maior número de folhas e comprimento da parte aérea na variedade Santa Luzia, na ausência de carvão. A variedade Thaís apresentou maior comprimento da parte aérea e da raiz na presença de carvão.

Termos para indexação: maná cubiu, micropropagação, cultura de tecidos vegetais, phytagel, Solanaceae.

Maná cubiu (*Solanum sessiliflorum* Dunal) is a fruit species belonging to the Solanaceae family and originating in Western Amazon. The fruit has nutritional and medicinal characteristics due to active principle niacin (vitamin B3), which plays a role in cell defense, besides being rich in fiber, phosphorus, iron, potassium, vitamin C, and pectin (Silva Filho et al., 2005).

The technique of micropropagation is one of the most practical applications of tissue culture and has a great impact, since large numbers of seedlings can be propagated using this method, and the crop can be obtained in a very short period of time (Dutra et al., 2009).

Solidifying agents are used in micropropagation because they provide ideal support conditions for plantlets, although the overall expenditure of in vitro production increases as these are expensive products. Agar and phytagel are the most commonly used solidifying agents in tissue culture. Agar is extracted from red algae, mainly from the species *Gelidium amansii* Lamouroux, and consists of a complex mixture of polysaccharides, especially agarose and agaropectins. Phytagel is a polysaccharide produced by the bacterium *Sphingomonas elodea* (ex. *Pseudomonas elodea*) and is composed of molecules of keto-glucuronate, rhamnose, and cellobiose (George, 1993). According to Babbar et al. (2005), the substitution of

agar with other polysaccharides, such as phytagel, improves the production of shoots and reduces the cost of micropropagation by up to 90%. Therefore, the use of alternate solidifying agents becomes important in order to increase the production of plants in vitro.

Activated charcoal is coal powder that has been finely milled in order to increase the adsorption area of the particles. Its addition to the culture medium promotes darkening of the medium. Moreover, it participates in the adsorption of substances released by the medium, such as the impurities in agar, growth regulators, organic compounds, or phenols released by the damaged tissues in vitro (George & Sherrington, 1984). Thus, activated charcoal is beneficial to the development of plants in vitro.

The objective of this work was to evaluate the effects of the solidifying agents agar and phytagel and of activated charcoal on the in vitro cultivation of two maná cubiu (*S. sessiliflorum*) varieties: Thaís and Santa Luzia.

The experiments were conducted at the plant tissue culture laboratory of the Department of Agriculture of Universidade Federal of Lavras (Ufla), in the municipality of Lavras, in the state of Minas Gerais, Brazil. Stem segments (bearing a 1-cm long bud) extracted from maná cubiu plants already established in vitro were used as explants.

For the evaluation of the effects of the solidifying agents, the basic culture medium used was MS (Murashige & Skoog, 1962) and the pH was adjusted to 5.8 before autoclaving (121°C and 1.0 atm for 20 min). For solidification of the culture medium, agar (HiMedia Laboratories, Mumbai, India) or phytagel (Sigma-Aldrich, Inc., St. Louis, MO, USA) was used. The concentrations used were: 5.5 g L^{-1} agar and 1.8 g L^{-1} phytagel; agar was used as a control treatment. The experiment was conducted in four replicates of three tubes each. The stem segments were inoculated in test tubes containing 15 mL of the culture medium and then maintained in a growth room with mean irradiance of 42 W m^{-2}, photoperiod of 16 hours, and temperature of 25±2°C.

The experimental design was completely randomized, in a 2×2 factorial arrangement, with two varieties of maná cubiu (Thaís and Santa Luzia) and two solidifying agents (agar and phytagel). After 60 days from the beginning of the experiment, the number of leaves, number of roots, shoot length, root length, mass of fresh shoot matter, and mass of fresh root matter of the plants were evaluated.

To evaluate the effect of activated charcoal, the MS culture medium was solidified using 1.8 g L^{-1} phytagel (using the same concentration as in the experiment on solidifying agents) and the pH was adjusted to 5.8 before autoclaving (121°C and 1.0 atm for 20 min). The concentrations of activated charcoal used were 0 g L^{-1} (control) and 2.0 g L^{-1}. The segments were inoculated in flasks containing 50 mL of the culture medium. After inoculation, the flasks were kept in a growth room with artificial light provided by special daylight fluorescent tubes, maintained at a mean irradiance of 42 W m^{-2}, photoperiod of 16 hours, and temperature of 25±2°C.

The experiment was performed in a completely randomized design, using a 2×2 factorial arrangement, activated charcoal (absence: 0 g L^{-1} and presence: 2.0 g L^{-1}), and maná cubiu varieties (Thaís and Santa Luzia), in six replicates. Each replicate consisted of a single flask containing two explants, which totaled to 12 explants per treatment. After 30 days from the beginning of the experiment, the following phytotechnical characteristics were evaluated: leaf number, number of roots, shoot length, root length, and fresh matter shoot and root.

The data obtained were subjected to the statistical software Sisvar (Ferreira, 2011), and the means were compared using Scott-Knott's test, at 5% probability.

Phytagel was superior to agar for all studied variables, independent by of the variety (Table 1). According to Chevreau et al. (1997), phytagel is highly purified and contains no foreign substances, whereas agar may contain substances that inhibit the development of plants in vitro. Chapla et al. (2009) also evaluated the effect of agar and phytagel on the in vitro growth of Miltonia flavescens Lindl. As in the present experiment, the authors obtained superior results when the plants were grown in a medium containing phytagel as the solidifying agent. Therefore, the use of purer solidifying agents promotes better results in in vitro culture.

In general, roots of the Thaís variety were longer than those of the Santa Luzia variety (Table 1). However, when the isolated effect of the solidifying agents was evaluated, it was possible to observe larger increments in root length in media containing phytagel. Chapla et al. (2009) also found longer root length in M. flavescens cultured in media containing phytagel, compared with agar.

In the absence of activated charcoal, shoots of the Santa Luzia variety were longer (Table 2). This result

Table 1. Phytotechnical characteristics of the maná cubiu (*Solanum sessiliflorum*) varieties Santa Luzia and Thais in medium containing the solidifying agents agar and phytagel[(1)].

Variety	Number of leaves		Shoot length (cm)		Fresh matter of shoots (g)		Fresh matter of roots (g)		Root length (cm)		
									Medium	Variety ME	
	Agar	Phytagel	Agar	Phytagel	Agar	Phytagel	Agar	Phytagel	main effect (ME)	Agar	Phytagel
Santa Luzia	6.2bB	10.7aA	1.7aB	11.9bA	0.133aB	0.775aA	0.022aB	0.131aA	10.9b	11.45B	15.42A
Thaís	7.7aA	8.4bA	3.0aB	6.0aA	0.227aB	0.473bA	0.021aB	0.092bA	16.0a		
CV (%)	15.96		31.19		29.49		49.84		32.62		

[(1)]Means followed by equal letters, lowercase in the columns and uppercase in the rows, do not differ significantly by Scott-Knott's test, at 5% probability.

Table 2. Phytotechnical characteristics of two varieties of maná cubiu (*Solanum sessiliflorum*) in the presence and absence of activated charcoal[(1)].

Activated charcoal	Number of leaves		Shoot length (cm)		Number of roots		Root length (cm)		Fresh matter of shoots (g)		Fresh matter of roots (g)	
	Santa Luzia	Thaís	Santa Luzia	Thaís	Santa Luzia	Thaís	Santa Luzia	Thaís	Santa Luzia	Thaís	Santa Luzia	Thaís
With	4.47bA	4.20aA	1.37bA	1.58aA	2.60bA	1.63bB	2.45bB	7.57aA	0.107bA	0.149aA	0.008bA	0.028bA
Without	5.10aA	4.03aB	3.13aA	1.80aB	4.03aA	2.37aB	9.91aA	7.90aA	0.341aA	0.184aB	0.161aA	0.102aB
CV (%)	16.15		45.66		42.5		60.22		81.02		90.93	

[(1)]Means followed by equal letters, lowercase in the columns and uppercase in the rows, do not differ significantly by Scott-Knott's test, at 5% probability.

corroborates with that of Unemoto et al. (2006), who evaluated the effect of the addition of activated charcoal on the culture medium and found that the absence of this product favored an increase in the shoot length of rainha-do-abismo [*Sinningia leucotricha* (Hoehne) Moore] plantlets. However, contrary results were found by Chapla et al. (2009), who observed longer shoots in media containing activated charcoal.

There was no significant difference in root length between the Thaís and Santa Luzia varieties in the absence of activated charcoal. However, the presence of activated charcoal promoted an increase in root length in the Thaís variety when compared with Santa Luzia (Table 2). Villa et al. (2007) also reported longer roots in 'Ébano' blackberry when activated charcoal was present in the culture medium.

Regardless of the variety studied, greater root length was observed in the absence of activated charcoal in the culture medium (Table 2). Chapla et al. (2009) also verified an increased root length in *M. flavescens* under the same conditions. Therefore, Maná cubiu showed better growth in vitro when the MS medium was solidified using phytagel, in the absence of activated charcoal.

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