Notas Científicas

Mannitol for coconut ex situ conservation by minimum growth

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Abstract – The objective of this work was to determine the mannitol concentration for in vitro germination and minimum growth of coconut (*Cocos nucifera*). The following accessions were used: Brazilian Red Dwarf Gramame (BRDG), Malaysian Yellow Dwarf (MYD), Brazilian Tall (BRA), Polynesian Tall (PYT), and Rennell Island Tall (RIT). Embryos were inoculated in an Y3 culture medium at different mannitol concentrations (0, 0.1, 0.2, 0.3, and 0.4 mol L^{-1}). Inhibition of shoot development was verified at 0.1, 0.2, and 0.3 mol L^{-1}, for MYD and BRDG, and at 0.1 and 0.2 mol L^{-1}, for PYT, BRA, and RIT. Mannitol use is a promising strategy for minimum growth conservation.

Index terms: *Cocos nucifera*, germplasm, osmotic regulator, tissue culture.

Manitol para a conservação ex situ de coqueiro por crescimento mínimo

Resumo – O objetivo deste trabalho foi determinar a concentração de manitol para a germinação e crescimento mínimo in vitro do coqueiro (*Cocos nucifera*). Os seguintes acessos foram utilizados: Anão-vermelho-do brasil-de-gramame (BRDG), Anão-amarelo-da-malásia (MYD), Gigante-do-brasil-praia-do-forte (BRA), Gigante-da-polinésia (PYT) e Gigante-de-rennel (RIT). Os embriões foram colocados em meio de cultura Y3 a diferentes concentrações de manitol (0, 0,1, 0,2, 0,3 e 0,4 mol L^{-1}). Verificou-se inibição do crescimento da parte aérea na presença de 0,1, 0,2 e 0,3 mol L^{-1}, para MYD e BRDG, e de 0,1 e 0,2 mol L^{-1}, para PYT, BRA e RIT. O uso do manitol é uma estratégia promissora para a conservação por crescimento mínimo.

Termos par indexação: *Cocos nucifera*, germoplasma, regulador osmótico, cultura de tecidos.

The conservation of the coconut (*Cocos nucifera* L.) germplasm is considered of worldwide importance and has resulted from the International Treaty on Genetic Resources for Food and Agriculture, organized by FAO, with 146 signatory countries, including Brazil (International treaty on plant genetic resources for food and agriculture, 2009). Coconut has a recalcitrant storage physiology and, therefore, the conservation of its genetic resources is primarily based on field collections (N'nan et al., 2012).

Several biotechnological techniques have been applied for genetic resources conservation, with emphasis on plant tissue culture and both short- and long-term in vitro conservation. In general, short-term storage is performed in studies focused on genetics and breeding programs, whereas long-term storage aims at genetic conservation (Engelmann, 2005). Additionally, in vitro collecting of germplasm can avoid the transmission of important coconut diseases, which do not pass through the embryo (Engelmann, 2005).

Osmotic regulators, such as sucrose and mannitol, act as growth retardants by causing osmotic stress to the material under conservation. When added to the culture medium, these carbohydrates reduce the hydric potential and restrict the water availability to explants (Shibli et al., 2006; Scherwinski-Pereira et al., 2010). Attempts to use in vitro conservation strategies for *C. nucifera* are restrict to a few studies. Lédo et al. (2007) observed minimum growth of the Brazilian Green Dwarf coconut accession (BGD) in the presence of mannitol. There are no results available for other Brazilian accessions, such as Brazilian Red Dwarf Gramame (BRDG) and Brazilian Tall (BRA).

The objective of this study was to determine the mannitol concentration for in vitro germination and minimum growth of coconut (*Cocos nucifera*).

The plant source used in this experiment was obtained from the International Coconut Genebank for Latin America and the Caribbean (ICG-LAC) (International Coconut Genetic Resources Network, 2013) coordinated by Embrapa Tabuleiros Costeiros and Coconut Genetic Resources Network (COGENT), located in Sergipe state, Brazil.

Mature fruit (11–12 months) of the coconut accessions Brazilian Red Dwarf Gramame (BRDG), Malaysian Yellow Dwarf (MYD), Brazilian Tall (BRA), Polynesian Tall (PYT), and Rennell Island Tall (RIT), were collected. Endosperm cylinders, containing zygotic embryos, were extracted from 250 fruit per accession, immersed in 2.5% (v/v) commercial sodium hypochlorite solution, and threefold washed in sterile water (Lédo et al., 2011). The cylinders were then placed in sterile containers and sent to the laboratory. The embryos were excised from the endosperm cylinders in aseptic conditions, immersed in 70% (v/v) ethyl alcohol for 2 min, then in a commercial solution of sodium hypochlorite (1% v/v) for 3 min, and threefold washed in sterile distilled water and placed in sterile Petri dishes (Lédo et al., 2011).

To evaluate mannitol effects on the in vitro germination and growth of coconut seedlings, the embryos were individually placed in test tubes (20x150 mm) with 15 mL Y3 culture medium (Eeuwens, 1976), supplemented with 0.3% (w/v) sucrose, 0.25% (w/v) activated charcoal, and gelled in 0.7% (w/v) agar and mannitol (0, 0.1, 0.2, 0.3, and 0.4 mol L^{-1}).

Culture media pH was adjusted to 5.8 before autoclaving (121°C and 1.0 atmosphere for 15 min). The cultures were maintained in growth chamber at 25±2ºC, 70% relative humidity, and in the absence of light until the induction of shoots (50 days after inoculation). After that, the cultures were maintained under a 12-hour photoperiod with approximately 38 µmol $s^{-1}m^{-2}$ photon lux. A completely randomized experimental design, in a 5x5 factorial arrangement with five replicates was used: five accessions, BRDG, MYD, BRA, PYT, and RIT; and five mannitol concentrations, 0, 0.1, 0.2, 0.3, and 0.4 mol L^{-1}. Fifty embryos were used in each treatment, totaling 250 embryos per accession.

Evaluations were performed for germination percentage, after 180 days in culture medium, and for shoot length of seedlings after 280 days of in vitro culture. Data were subjected to analysis of variance and means were compared by Tukey's test, at 5% of probability. For mannitol concentrations, data were evaluated by regression analysis.

Accessions and mannitol concentrations had significant effects on germination and shoot length of seedlings. Accessions differed as to their germination capacity in the presence of mannitol (Table 1). Mannitol concentrations had a quadratic effect on germination of tall accessions (Figure 1). For dwarf accessions, there was no difference on germination percentage.

The accessions MYD and BRDG of dwarf coconut had 100% germination, either in the presence or in the absence of mannitol. These results do not agree with those of Karun et al. (2002), who did not observe germination for Tall West Coast, Tall Laccadive, and Chowghat Orange Dwarf embryos in the presence of mannitol. As for tall accessions, BRA had a lower germination than RIT and PYT in the presence of 0.1 and 0.2 mol L^{-1} mannitol, and no germination at 0.3 mol L^{-1}. Therefore, this accession was the most sensitive to osmotic stress. This variation on germination percentage of the accessions is probably linked to genetic effects. The underlying molecular mechanisms involved in the adaptation of seed germination to water restrictions and salt excesses have been postulated (Vallejo et al., 2011). The BRA and RYT accessions did not germinate at 0.4 mol L^{-1} and, therefore, it was not possible to study the effects of this concentration on the shoot length.

Table 1. In vitro germination percentage of zygotic embryos of Rennell Island Tall (RIT), Brazilian Tall (BRA), Polynesian Tall (PYT), Brazilian Red Dwarf Gramame (BRDG), and Malaysian Yellow Dwarf (MYD) coconut accessions, at different concentrations of mannitol in 180 days[(1)].

Mannitol	Accessions				
(mol L^{-1})	RIT	BRA	PYT	BRDG	MYD
0.0	100.00a	88.88ab	70.00b	100.00a	100.00a
0.1	77.77b	55.55c	60.00b	100.00a	100.00a
0.2	77.77b	55.55c	80.00b	100.00a	100.00a
0.3	55.55b	00.00c	50.00b	100.00a	100.00a
0.4	00.00c	00.00c	20.00b	100.00a	100.00a
VC (%)			14.02		

[(1)]Means followed by equal letters in the lines do not differ at 5% probability, according to Tukey's test. Tall accessions: RIT, BRA, and PYT. Dwarf accessions: BRDG, MYD.

Mannitol also had significant effect on shoot length of seedlings, both from tall and dwarf accessions (Figure 1), at 280 days of in vitro culture. Shoot length decreased with increasing concentrations of mannitol, with linear tendency for RIT, BRDG and MYD accessions, and quadratic for BRA and PYT.

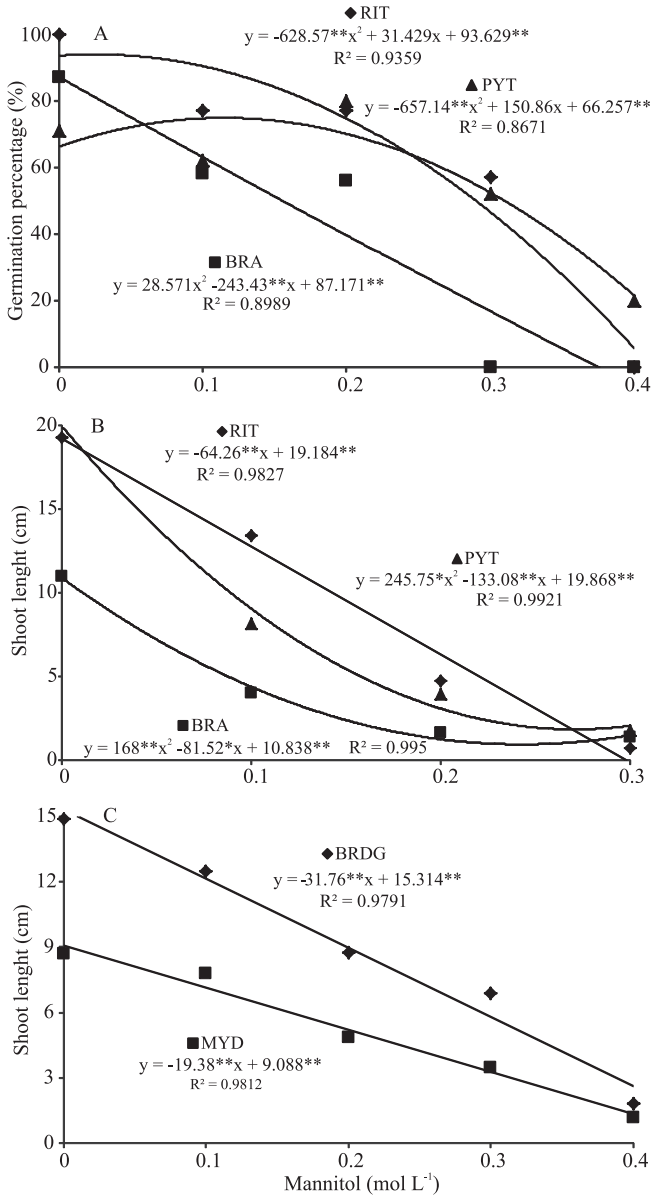

Figure 1. In vitro germination percentage of zygotic embryos (A), shoot length of seedlings from tall coconut accessions RIT, BRA, PYT (B), and shoot length of seedlings from dwarf coconut accessions BRDG and MYD (C), at different concentrations of mannitol. Brazilian Red Dwarf Gramame (BRDG), Malaysian Yellow Dwarf (MYD), Brazilian Tall (BRA), Polynesian Tall (PYT), and Rennell Island Tall (RIT).

Depending on the concentration or on the species, mannitol can cause deleterious effects on shoot growth (Shibli et al., 2006; Silva & Scherwinski-Pereira, 2011). The concentration of 0.4 mol L^{-1} was deleterious for shoot development. The inhibitory effect on BRDG growth was evident from 0.2 mol L^{-1}, with deleterious effect at 0.4 mol L^{-1}, leading to the wilt and death of seedlings. Similar results were observed by Lédo et al. (2007), who have reported that the addition of mannitol at 0.3 or 0.4 mol L^{-1} promotes slower growth of shoots and roots, and leads to a lower survival of seedlings of the BGD accession.

Mannitol concentration at 0.1 mol L^{-1} is recommended for RIT, BRA, PYT, and MYD, and at 0.1 or 0.2 mol L^{-1} for BRDG accession, for in vitro germination and minimum growth.

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