Development of *in vitro* conservation protocols in palms, using *Bactris gasipaes* Kunth ('chontaduro') as a model.

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Introduction

Bactris gasipaes belongs to the Palmae family that comprises 64 species distributed in tropical America, and is known as chontaduro, admirable palm, pijuayo, pupunja or pejibaye. It has been identified as a promising crop with several uses: its fruits for direct human consumption because of its nutritional value, as flour and meal, oil, and as heart of palm. Some difficulties in relation to conservation have been evidenced such as: parthenocarpy and recalcitrant seeds, xenogamy and autoincompatibility (Mora & Solis, 1980).

The use of chontaduro as model of study is justified because it is one of the most representative species in Colombia, for the increasing demand of its products. In addition, two varieties: Chontaduro (*B. gasipaes* var. *gasipaes*) and Chinamato (*B. gasipaes* var. *chichagui*) are reported in the Red List of Phanerogams of Colombia (Bernal & Galeano, 2004). There are futher prospects to establish methodologies applicable to materials of African palm (*Elaeis guineensis* Jacq.) and other species reported in the Red List.

In vitro germplasm conservation based on conditions that permit minimal rates of growth, for instance reducing the temperature or using growth retardants, has been used for many species. In our study we proposed two objectives: 1) to establish conditions of management for cryoconservation of chontaduro, and 2) to establish a method of *in vitro* conservation of *B. gasipaes* under minimal slow growth.

Materials and Methods

1) Cryopreservation of chontaduro

The seeds we used came from mature fruits (four months after the pollination), obtained from a farmer in Armenia, Colombia. Six moisture content (MC) levels were tested (4, 8, 10,12, 20 and 40 %); these levels were reached in dry rooms (20 ºC and 40% RH) and the drying curve is presented in Figure 1 . The seeds were then vacuum packed in aluminum foil bags (10 seeds per package for each MC). The bags were placed in liquid nitrogen (-196 °C) and remained for 7 and 30 days under these conditions. This test was repeated twice. The packages of seed were extracted from the liquid nitrogen, defrosted slowly, and viability and regeneration were evaluated using rescue of embryos (Figure 2).

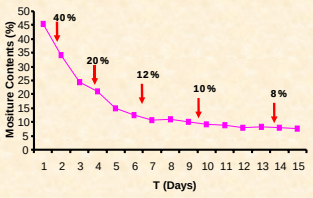

Figure 1. Drying curve for seeds of *B. gasipaes* K. placed at 20°C and 40% of relative humidity.

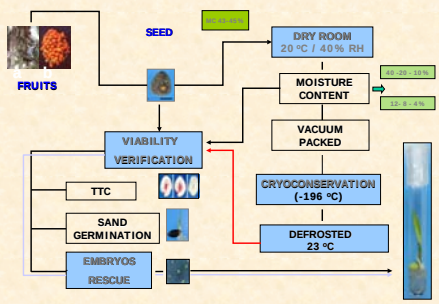

Figure 2. Steps in the development of cryopreservation procedures for chontaduro.

2) Methods of *in vitro* conservation of *B. gasipaes* under minimal slow growth.

Two conditions of temperature and two culture media were evaluated (28°C and 24°C/ SN and 8S) for minimal slow growth. The cultures were incubated under a 12-hours photoperiod (1,000 lux). Each treatment included 10 replicates. After 8 months of storage, observations were recorded on stem length (mm), number of leaves and roots.

Results and discussion

1) Cryopreservation of chontaduro

We observed 100% of plants regenerated from seeds with 40% MC (control), while for seeds with a 10 and 20% MC the averages were 50 and 48%, respectively. The previous result indicates that after lowering the percentages of MC of the seed, the percentage of regeneration of *in vitro* plants goes down due to possible damages caused in the structures of the embryo that do not allow its subsequent germination (Table 1).

Table 1. Means of viability and regeneration *in vitro* of seeds of chontaduro for two times of conservation in liquid nitrogen (-196 °C).

Time NL (days)	Moisture content (%)					
	10		20		40	
	%Via.	% Reg.	%Via.	% Reg.	%Via.	% Reg.
0 (control)	64 b	48 b	70 b	50 b	100 a	100 a
7	80 b	63 b	0 c	0 c	0 c	0 c
30	80 b	53 b	0 c	0 c	0 c	0 c

Viab. CME **0.02** – **Regen.** CME **0.01** DF **22**

The analysis showed statistical differences for the percentage of regeneration and *in vitro* viability in the interaction of the two principal effects: MC (%) and time of storage in NL (days). For the embryos extracted from cryopreserved seeds with 20 and 40 % MC, there was no response in viability nor *in vitro* regeneration, whereas at 10 % of MC we registered percentages of viability of 80 % after 7 and 30 days of conservation and percentages of regeneration of 63 and 53 %, respectively. These results suggest that moisture contents higher than 20 % are not appropiate for the cryopreservation of seeds of chontaduro (Figure 3).

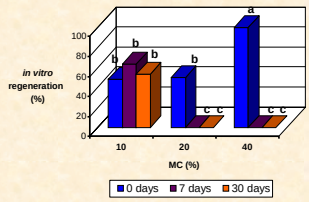

Figure 3. Effect of the cryopreservation in NL on the regeneration of seeds of *B. gasipaes* for three moisture contents (10, 20 and 40%).

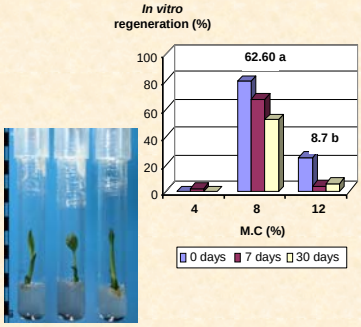

Figure 4. In vitro regeneration (%) of seeds of *B. gasipaes* cryopreserved at three MC (4, 8 and 12%) for three durations (0, 7 and 30 days).

With the purpose of adjusting the ideal MC for the cryopreservation of chontaduro, new ranges of contents of moisture were evaluated. The MC of seed evaluated in this second phase were 12, 8 and 4 %, for which we determined viability and *in vitro* regeneration. The results show that a reduction in the MC of the seeds down to 4 % turns out to be too drastic.

For the seeds in liquid nitrogen with 8 % MC we registered high percentages of viability (80 %) and regeneration (60 %) for two times of conservation. In contrast, the seeds with 12 % MC had low answers for these variables (9 % viability and 5 % regeneration) (Figures 4). These results indicate that the ideal MC is 8%.

It is expected that these protocols are not genotype dependent so that their application could be possible to a wide range of accessions maintained in field collections in Colombia.

2) *in vitro* conservation of *B. gasipaes* under minimal slow growth

The analyses of variance showed that our conservation conditions (24°C and 28°C) had a greater effect on stem elongation and number of green leaves, while no significant differences were observed between the different medias. A reduction of growth was obtained when the material remained at a temperature of 24°C, indicating that the physical factor (temperature) has an effect on the *in vitro* growth of chontaduro. (Figure 5).

For the material maintained at a temperature of 24 °C in the 8S media, it was possible to observe yellowing in the leaves, indicating the beginning of a possible process of deterioration; this response was not observed in the *in vitro* plants grown in SN (silver nitrate) media.

It is important to emphasize that after eight months of evaluation of the media and conditions of conservation we have not seen senescence. Avoiding or lowering down senescence is an important factor to consider when evaluating conservation under minimal growth.

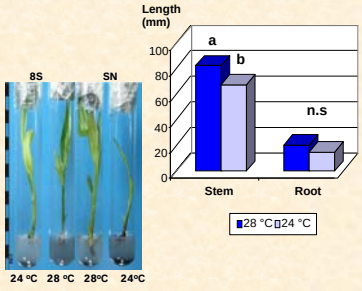

Figure 5. Effect of temperature on stem elongation and length of roots (mm) for the in vitro conservation of *B. gasipaes* (time of conservation: eight months).

Conclusions

This experiment constitutes the first report of successful cryopreservation of seeds of a member of the genus *Bactris*. Seeds of *B. gasipaes* seem to tolerate the drying down to 8% of MC showing high percentages of viability (80%) and regeneration (62.6%) for the times of cryoconservation evaluated. The method of cryoconservation of seeds developed in this research can be used as a strategy for the conservation of species of palms reported in the Red List of Phanerogams of Colombia.

After 8 months of conservation in vitro a decrease in the temperature has allowed to reduce the rate of growth of the plants of *B. gasipaes*. Media with silver nitrate have not shown an effect on the decrease in the rate of growth, however we observed that the number of leaves and their color has been slightly higher than those of the media 8S. In the medias and under the evaluated conditions of temperature, we observed 95% of survival.

Acknowledgements

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