



Cryopreservation Techniques of Plumules of Brazilian Green Dwarf Coconut Accession (BGD)

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INTRODUCTION

The coconut has a recalcitrant storage physiology, therefore the conservation of genetic resources of this perennial crop is primarily based on field collections. The establishment of alternative strategies for the ex situ conservation is more important for improved the genetic resource and breeding programs for this palm. Several authors have published promising results with zygotic embryo, plumules and pollen. The objective was to evaluate the effect of cryoprotectant solutions and immersion times on the survival and regeneration of Brazilian green dwarf (BGD) accession.

MATERIAL AND METHODS

Vegetal Material: plumules excised from mature zygotic embryos of Coconut fruits (10-11 months) of Active Germplasm Bank of Embrapa Coastal Tablelands, Sergipe, Brazil.

Cryopreservation Techniques: Encapsulation-dehydration and droplet-vitrification

Pre-culturing: The plumules, after the disinfection, were initially precultured for 72 hours in Y3 medium with 0.12 M sucrose.

Encapsulation-dehydration: were formed beads with the plumules. Beads were transferred to cryoprotectant solutions composed by Y3 medium supplemented with 0.5 or 1.0 M sucrose and incubated on rotary shaker providing gentle shaking for 48 hours at $25 \pm 2^\circ$ C in the absence of light.

Half of the beads was then transferred to a recovery medium (-LN), while the other half of the beads was placed in 2 mL sterile cryogenic vials and rapidly cooled by direct immersion into liquid nitrogen (+LN).

Droplet-vitrification: the explants were immersed in the PVS2 vitrification solution for 30, 45 and 60 minutes. After dehydrating the plumules were transferred to aluminum foil and introduced into sterile polypropylene cryotubes and quickly immersed in +LN.

Thawed: in a water bath at $38 \pm 2^\circ$ C for 2 to 3 minutes and then cultured on regeneration medium.

Evaluations: After 120 days, percentage of survival and induction of somatic embryogenesis (-LN and +LN) .

CONCLUSION

The cryoprotectant solutions composed through Y3 medium supplemented with 0.5 or 1 M sucrose provide 86.67 and 66.67%, respectively, of survival in plumule encapsulated. The immersion in PVS2 solution for 30 minutes promoted 100% survival. Both methods can be recommended for future BGD accession coconut cryopreservation protocols.

RESULTS

Table 1. Survival and recovery rate of BGD coconut plumule beads in function of cryoprotectant solutions, 120 days.

cryoprotectant solutions	Survival (%)		
	Y3 Medium + 0.5 M Sucrose	Y3 Medium +1 M Sucrose	
LN -	100.00	100.00	100.00A
LN +	69.23	54.54	62.50B
	86.67a	66.67a	
VC (%)	41.70		
cryoprotectant solutions	Recovery (%)		
	Y3 Medium + 0.5 M Sucrose	Y3 Medium +1 M Sucrose	
LN -	46.15	36.60	41.67A
LN+	5.88	0	4.76B
	23.33a	26.67a	
VC(%)	26.00		

Means followed by the same capital case letters in a column and lower letters on the lines do not differ significantly by the Tukey test ($p < 0.05$).

Table 2. Survival and recovery rate of BGD coconut plumule in function of the immersion time in PVS2, 120 days.

Immersion (minutes)	Survival (%)	Recovery (%)
30	100.00A	0
45	43.00B	0
60	27.00B	0
VC (%)	63.02	

Means followed by the same capital case letters in a column do not differ significantly by the Tukey test ($p < 0.05$).



Recovery by somatic embryogenesis of BGD coconut plumules (Y3 Medium + 50 mg L⁻¹ 2,4-D).