

INDUCTION OF GROWTH IN FROZEN EMBRYOS OF COCONUT AND OVULES OF CITRUS

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THE storage of seeds is the customary method for conservation and international exchange of germplasm. However, in a number of tree species and plantation crops the seeds are recalcitrant¹. Such seeds are sensitive to changes in humidity and temperature, and thus cannot be preserved under ordinary conditions for long periods, due to degeneration of embryos. In such cases, the germplasm could possibly be conserved through the cryopreservation of embryos or their segments^{2,3}. In this communication survival of frozen embryos of two tree species (coconut and citrus), whose seeds are short-lived, is reported.

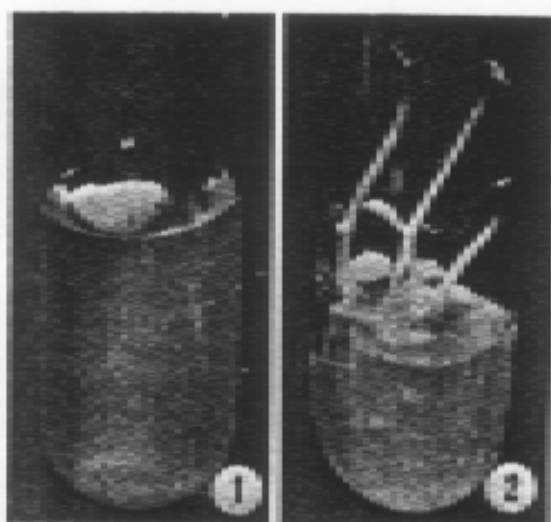
The immature embryos (1–1.5 cm) of West Coast Tall cultivar of coconut (*Cocos nucifera* L.) removed from nuts and stored for one month were partially dehydrated, and were cut into transverse halves. They were treated with a cryoprotectant solution (7% dimethylsulfoxide and 7% sucrose in Murashige and Skoogs' liquid medium⁴), blotted dry, then wrapped in a single layer of sterile aluminium foil⁵, and frozen by gradually lowering into the liquid nitrogen cylinder, or were suddenly dropped in it and kept for five minutes. The frozen material was then thawed in warm water (35–40°C), washed and cultured on MS + 2,4-D (0.2 mg/l) + NAA (0.5 mg/l) + kinetin (0.1 mg/l). Likewise, the young ovules taken from the unripe fruits of *Citrus* sps. were subjected to the same protocol, and cultured on a medium supplemented with casein hydrolysate (CH)⁶.

The retrieved coconut embryos and their segments in cultures showed a lag period of upto 4 months without showing any sign of growth. However, in some of the cultures, the embryos subsequently showed an overall swelling and elongation. The embryo segment at the cut end underwent sparse proliferation (figure 1, table 1), which at places turned brown.

Entire young ovules, and the micropylar half of the split citrus ovules showed a survival of 28.8 and 24.3% respectively (table 1). The retrieved material, like the controls⁷ when cultured on a medium containing CH (500 mg/l), proliferated to form pseudobulbils⁸. Figure 2 shows the shoots obtained from the frozen ovules.

Table 1 Survival of embryos of coconut and ovules of citrus subjected to freezing in the presence of DMSO (7%) + sucrose (7%)

Crop	Explant	No. of cultures frozen	No. of cultures showing signs of growth	% survival	Remarks
Coconut	1. Embryo (1-1.5 cm)	17	3	17.6	Embryo elongation Proliferation at the cut end
	2. Transverse halves of embryo	28	7	25.0	
Citrus	1. Entire ovule	59	17	28.8	Proliferation to form callus and shoots
	2. Micropylar half of the ovule (with nucellar embryos)	37	9	24.3	



Figures 1, 2. 1. A 4-month-old retrieved culture of a transverse half of a coconut embryo; note the overall swelling, and proliferation at the cut end (right side). 2. Shoot formation from frozen ovules of citrus 4 months after culture on CH (500 mg/l) medium.

The survival, and the induction of growth in coconut embryos frozen at -196°C points to the possibility of the long-term conservation of germ-plasm of this plantation crop with large-sized recalcitrant seeds.

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