

## Cryopreservation of encapsulated plumules of coconut: effect of transport/store conditions

H D D Bandupriya<sup>1\*</sup>, S C Fernando<sup>1</sup>, J-L Verdeil<sup>2</sup> and B Malaurie<sup>3</sup>

<sup>1</sup>Coconut Research Institute, Lunuwila, Sri Lanka.

<sup>2</sup>CIRAD, TA40/02 Avenue Agropolis, 34398 Montpellier Cedex 5 – France.

<sup>3</sup>UMR 1098 BEPC, Institute for Research and Development (IRD), BP 64501, 911 Av Agropolis 34394, Montpellier, France

Proceedings Asia Pacific Conference on Plant Tissue and Agribiotechnology (APuCPA) 17-21 June 2007

**Abstract.** Attempts were made to identify the most suitable method to transport/store mature zygotic embryos of coconut (for excision of plumules later on) for cryopreservation work. Three different conditions, embryos transported/stored in albumen (solid endosperm) cores, solidified agar [0.45 % (w/v) agar] and KCl solution (16.2 g/L) were tested. Encapsulation/dehydration method was employed for cryopreservation. In this method, the plumules were excised from mature zygotic embryos (transported/stored under the 3 conditions indicated above) and encapsulated in sodium alginate. The beads were then pretreated with 0.75 M or 1.0 M sucrose. Prior to freezing in liquid nitrogen (LN), the plumules were subjected to further desiccation (D) by exposure to silica gel for 16 h. The condition used to transport/store embryos prior to excision of plumules has a significant effect on recovery of dehydrated but unfrozen plumules (+D/-LN) that were pretreated with 0.75 M sucrose. The plumules excised from embryos stored in KCl and solidified agar showed a significantly higher rate of recovery when compared to albumen cores. However, in frozen plumules (+D/+LN), there was no significant difference in recovery under 3 conditions tested. In frozen plumules (+D/+LN) pretreated with 1.0 M sucrose, the rate of recovery (40%) was significantly higher in the ones excised from embryos stored in solidified agar when compared to the other two conditions. However, this difference is not reflected in unfrozen plumules (+D/-LN).

**Keywords:** Cryopreservation; Plumule; Coconut; Embryo storage.

### INTRODUCTION

Coconut genetic resources are the reservoir of genes essential for crop improvement so as to develop varieties adapted to socio economic requirements and changed environments. The coconut genetic diversity is how ever diminishing at a high rate due to various environmental and human directed factors. Conservation in the field gene banks requires huge land areas and is very expensive. Thus, Cryopreservation is an important alternative method for long-term conservation of coconut germplasm.

Successful cryopreservation of coconut embryos from immature nuts (7-8 months after pollination) and mature nuts has been reported (Assy-Bah and Engelmann, 1992a, b). Inadequate recovery conditions led to the low recovery of plantlets inspite of high survival rates obtained after cryopreservation. Coconut plumule (embryo meristem and first leaves), which is known to be free of viral diseases, appears to be an interesting starting material for cryopreservation (Maularie et al., 2002). Cryopreservation of plumules is vital because of their small size (around 1mm) and the structure in which lot of meristematic cells can be observed. Coconut plumules have been cryopreserved by encapsulation/dehydration method using different sucrose concentrations and dehydration periods, resulting in 40-70% survival after cryopreservation (N'Nan, 2004; Malaurie et al., 2002).

The cryopreservation protocol needed to be perfected

for introduction and exchange of selected populations of coconut for the crop improvement programmes of the host country as well as the other countries in the same region. Coconut seeds are bulky and heavy, thus making them costly to transport. Coconut embryo has been adapted by various researchers for germplasm exchange. The simplest procedure (Rillo and Paloma, 1991) consists of collecting endosperm plugs. Other protocols (Assy-Bah et al., 1987; Karun, 2001) involve disinfection and in vitro inoculation of embryos in a growth medium.

In the present study, attempts were made to identify the most suitable method to transport/store mature zygotic embryos of coconut (for excision of plumules later on) for cryopreservation work using the optimized conditions (sucrose concentration and dehydration duration) reported by N'Nan, 2004.

### METHODS AND MATERIALS

Mature nuts were obtained from 12 to 14 month old nuts of the variety Sri Lanka Tall. Three different transport/store conditions were tested. In the first method, extracted solid

\* Author for correspondence: Research Institute, Lunuwila, Sri Lanka.  
Email: dbandupriya@yahoo.com

endosperm plugs were washed in tap water and in 95% ethanol quickly to remove the fats, and then disinfected with 100% commercial bleach for 20 minutes. The plugs are then washed with sterile water and packed in sterile polypropylene bags and refrigerated for 10 days. In the second and third methods, coconut embryos were disinfected with 20% commercial bleach for 5 minutes, rinsed with sterile water, cultured in solidified agar [0.45% (w/v)] and KCl solution (16.2 g/L) respectively and then stored in at 27°C in dark for 10 days. After the storage period, the endosperm plugs were re-sterilized in 100% commercial bleach for 20 minutes and embryos were dissected inside a laminar air flow cabinet. The embryos dissected from the above three methods were then disinfected with 20% commercial bleach for 5 minutes and then washed with sterile water for 5 times.

The plumules were excised from the embryos in the laminar airflow cabinet under a stereo microscope and pre-cultured in Eeuwens Y3 growth medium (Eeuwens, 1976) for 4-7 days. Then, the encapsulation/dehydration was done as describe by Malaurie et al 2002. For the sucrose pretreatment liquid Eeuwens medium enriched either 0.75 M or 1.0 M sucrose was used. The dehydration was carried out for 16 h. Half of the dehydrated beads were transferred to sterile polypropylene cryotubes and directly plunged in to liquid nitrogen. After a minimum of 2 hours storage in liquid nitrogen, tubes were rewarmed inside a water bath at 40 °C for 3 min. Then the individual beads were cultured in recovery medium (Eeuwens Y3 growth medium). The remaining, unfrozen beads (subjected to pretreatment and desiccation) were also cultured in the same growth medium.

After two months of culture, the survival was assessed as the percentage plumules manifesting new tissue growth (indicated by any sign of regrowth such as swelling, development of new leaf primordia and/or callus formation). The recovery of plumules (indicated by the ability of plumules to grow in to plantlets) was assessed after 4 months in culture. The experiments were arranged in a complete randomized design with two replicates of 10 plumules per treatment.

## RESULTS AND DISCUSSION

In coconut, a highly recalcitrant seed species, successful cryopresevation has been reported from immature nuts (7-8 months after pollination) and mature nuts (Assy-Bah and Engelmann, 1992a, b). How ever, mature embryos withstood cryopresevation better as judged by recovery of whole plants after freezing (Assy-Bah and Engelmann, 1992, b). From these results it was revealed that only the meristematic zone where the shoot and root pole exists, could withstand exposure to liquid nitrogen. Based on these observations new cryopreservation protocols were worked out on plular tissues.

Identifying a safest method for international exchange of coconut germplasm that is free of known diseases is a pre-request for cryogenic storage. This is also essential for

duplicating germplasm collections. The condition used to transport/store embryos prior to excision of plumules has a significant effect on recovery of dehydrated but unfrozen plumules (+D/-LN) that were pretreated with 0.75 M sucrose (figure 1). The plumules excised from embryos stored in KCl and solidified agar showed a significantly higher rate of recovery when compared to albumen cores. However, in frozen (+D/+LN) plumules pretreated with 0.75 M sucrose; there was no significant difference in recovery under 3 conditions tested. As shown in figure 2, in frozen plumules (+D/+LN) pretreated with 1.0 M sucrose, the rate of recovery (40%) was significantly higher in the ones excised from embryos stored in solidified agar when compared to the other two conditions. However, this difference is not reflected in unfrozen plumules (+D/-LN).

With regard to recovery of both frozen and unfrozen embryos, the positive effect of storing them in solified agar was quite clear. The preliminary results obtained from this investigation are quite promising and further experiments could be based on these results. Further refinements to the procedures developed in this study could lead to a reliable and efficient cryopreservation protocol, which could be applied for long term storage of coconut germplasm.

## ACKNOWLEDGEMENTS

Authors gratefully acknowledge the financial assistance provided by the IRD, through a research project funded by Bureau des Ressources Genetiques, Paris, France (BRG). Thanks are also due to all staff members of Lab Genetrop, IRD, France and Tissue Culture Division, CRI, Sri Lanka for their assistance in carrying out the experiments.

## REFERENCES

- Assy-Bah, B. and Engelmann, F. 1992a. Cryopreservation of immature embryos of coconut (*Cocos nucifera* L.). *Cryo-letters* 13: 67-74.
- Assy-Bah, B. and Engelmann, F. 1992b. Cryopreservation of mature embryos of coconut (*Cocos nucifera* L.) and subsequent regeneration of plantlets. *Cryo-letters* 13: 117-126.
- Assy-Bah, B., Durand-Gasselien, T. and Pannetier, C. 1987. *In: Use of zygotic embryo culture to collect germplasm of coconut (Cocos nucifera L.)*. Plant Genetic Resources Newsletter 71: 4-10.
- Eeuwens, C. J. 1976. Mineral requirements for growth and callus initiation of tissue explants excised from mature coconut palm (*Cocos nucifera*) and cultured *in vitro*. *Plant Physiology*. 36: 23-28.

- Karun, A. 2001. Embryo culture protocol for collection and exchange of coconut germplasm. *Indian Coconut Journal-Cochin* 31: 17-18.
- Malaurie, B. and Borges, M. 2001. *In: Cryopreservation of coconut (Cocos nucifera L.) plumules by encapsulation/dehydration. BIOVEG 2001. International Workshop on plant Biotechnology. Centro de Bioplasmas, Ciego de Avila, Cuba, Short Report Abstract p59.*
- Malaurie, B., N'Nan, O., Hocher, V., Ilbert, P., Grosdemang, F., Konan, J-L., Zakra, N., and Verdeil, J-L. *In: State of zygotic coconut embryo culture and cryopreservation research at IRD/CIRAD (France) in Engelmann F, Batugal P and Oliver J T (eds) 2002. Coconut Embryo in vitro Culture Part II, IPGRI-APO, Serdang, Malaysia.*
- N'Nan, O. 2004. *In: Utilisation des biotechnologies comme seconde voie pour les échanges et la conservation des ressources génétiques du cocotier (Cocos nucifera L.), PhD thesis, Angers University.*
- Rillo, E.P. and Paloma, M. B. 1991. *In: Storage and transport of zygotic embryos of Cocos nucifera L. for in vitro culture. FAO/IPGRI Plant Genetic Resources Newsletter 86: 1-4.*

Bandupriya H.D.D., Fernando S.C., Verdeil J.L., Malaurie Bernard (2010)

Cryopreservation of encapsulated plumules of coconut :  
effect of transport/store conditions

Asian Pacific Journal of Molecular Biology and Biotechnology,  
18 (1), 135-137