

Kamlai Reanhatthakam 2011: Optimization of Cryopreservation Technique of Rattan's Embryo Master of Science (Agricultural Research and Development), Major Field: Agricultural Research and Development, Faculty of Agriculture at Kamphaeng Saen Thesis Advisor: Associate Professor Sontichai Chanprame, Ph.D. 89 pages.

Cryopreservation technique for rattan embryo was studied as it would be used as a guideline for rattan genetic conservation. Three rattan species, *Calamus* sp., *C. longisetus* Griff. and *C. myrianthus* Becc., were *in vitro* cultured at plant tissue culture laboratory, RSPG. Three methods of cryopreservation vitrification, encapsulation-dehydration and encapsulation-vitrification, were studied. The survival percentage of embryo before liquid nitrogen immersion was 100% and after liquid nitrogen immersion were 20 80 and 100% in *Calamus* sp., *C. longisetus* Griff., *C. myrianthus* Becc, respectively for encapsulation-dehydration method. The most suitable protocol was preculturing the embryos on preculture medium for seven days. The embryos were then encapsulated and left in loading solution containing 0.8M sucrose and 1M glycerol for 0 20 or 30 minutes. The encapsulated embryos were then dehydrated using silica gel (20 embryos per 50 g silica gel) for 14 or 21 hours. After immersing in liquid nitrogen, the encapsulated embryos were thawed at 37-40 °C for 2 minute and left in unloading solution containing 1.2 M sucrose for 20 minute. After reculturing on MS + 0.3 M sucrose for 1 day, the embryos were taken out from supported medium and transferred onto MS medium . They were then incubated for 1 month. The same technique was also applied to preserve another 4 rattan species in liquid nitrogen; *Daemonorops brachystachys* Korthalsia *grandis* Rild. *C. tenuis* Roxb. and *C. pandanosmus* Furt.. The survival percentage of embryos after liquid nitrogen immersion were 80 80 80 and 70% respectively. Genetic stability of 2 species of rattan embryo (*Calamus* sp. and *C. tenuis* Roxb.) preserved in liquid nitrogen was also studied using AFLP technique. A few polymorphic DNA band (3%) were found in *C. tenuis* Roxb. While non polymorphic DNA band was found in *Calamus* sp.

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Thesis Advisor's signature