

**RESEARCH OF AN OPTIMAL CRYOPRESERVATION PROCESS USING
ENCAPSULATION/ OSMOPROTECTION/ DEHYDRATION AND
ENCAPSULATION/OSMOPROTECTION /VITRIFICATION TECHNIQUES ON
CAULINARY MERISTEMS OF COCONUT (*COCOS NUCIFERA* L.).**

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Cryopreservation were applied to plumular tissues excised from MYD coconut (*Cocos nucifera* L.) zygotic embryos using encapsulation/osmoprotection /dehydration and

encapsulation/osmoprotection/vitrification techniques as describe by Sakai *et al.* (2000). Plumules were cultured on 0.3M sucrose before their encapsulation in alginate solution added with osmoprotectants: glycerol and sucrose. Dehydration of the beads were done using silica gel and conduct every hour from 2h to 10h. In the case of encapsulation/osmoprotection/vitrification treatments were similar until the end of osmoprotection, where dehydration is replaced by vitrification step with the use of PVS2 solution during 20, 60 and 100mn. Direct freezing of the beads, placed into cryovials, were realized by their rapid immersion into liquid nitrogen (LN) (-196°C). After rapid thawing, beads were cultured on standard medium without growth regulators, and growth observed after two months culture. The influence of dehydration duration on pre and post freeze plumular growth were examined. Various morphogenetic factors such as: swelling, shooting, and growth of the gemmule were measured. Results were evaluated from three replicates. For the first technique, swelling was observed in all the treatments with a mean of 90%. Whereas, in 3h dehydration treatment shooting could be obtained either with and without liquid nitrogen, up to 25% or 23% respectively. Increase in length of the plumule varied from 1 to 1,5mm (+LN), compared 1,2 to 3,8mm (-LN). Plumular tissues still remain affected by dehydration and (LN) with mean up to 79% of browning. In the later technique, 100% browning was observed for (+LN), whereas in (-LN) the browning increased from 14 to 65% in PVS2 similar to dehydration duration. Swelling and shooting were observed only in (-LN). Swelling reached a mean of 96% with three dehydration duration, whereas shooting was observed only after 20mn in a rate of 11%.

Malaurie Bernard, Borges M., N'nan Oulo (2002)

Research of an optimal cryopreservation process using encapsulation/osmoprotection/dehydration and encapsulation/osmoprotection/vitrification techniques on caulinary meristems of coconut (*Cocos nucifera* L.)

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