

## CRYOPRESERVATION OF SHOOT TIPS OF *Trichilia emetica*, A TROPICAL RECALCITRANT-SEEDED SPECIES

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### Abstract

This paper reports the successful cryopreservation of shoot tips of *Trichilia emetica*, a tropical tree species producing recalcitrant seeds. Preculture of shoot tips on MS medium with 0.7 M sucrose or with 0.3 M sucrose + 0.5 M glycerol followed by cryoprotection with a mixture of glycerol and DMSO or with PVS2 was crucial for successful recovery following cryostorage. Three cooling rates were applied to assess the effects on post-thaw regrowth of shoot tips. Slow cooling of the shoot tips (WC 1.24 g g<sup>-1</sup> DW) precultured on medium with 0.3 M sucrose + 0.5 M glycerol and cryoprotected with PVS2 resulted in high shoot production (71%). Subsequent to relatively faster cooling, only 38% of the shoot tips developed shoots. Ultra-rapid cooling with PVS2 resulted in callus formation with 55% regrowth. We report one of the very few successful attempts to cryopreserve explants alternative to zygotic axes of tropical tree species producing recalcitrant seeds.

**Keywords:** cooling rates, cryopreservation, preculture, shoot tips, vitrification

**Abbreviations:** BAP: 6-benzylaminopurine; DMSO: dimethyl sulphoxide; DW: dry weight; GA<sub>3</sub>: gibberellic acid; LN: liquid nitrogen; MS: Murashige & Skoog medium; PVS2: plant vitrification solution 2; WC: water content; WPM: woody plant medium

### INTRODUCTION

Many African plant species produce short-lived, recalcitrant (desiccation-sensitive) seeds (33). The only option for long-term conservation of the genetic resources of such recalcitrant-seeded species is by cryostorage – generally in LN at -196°C or in LN vapour (-150 to -160°C). Excised embryonic axes of such seeds should be amenable to cryopreservation and hence are theoretically ideal explants (17, 38). However, in several cases, embryonic axes of mature recalcitrant seeds are themselves large structures (e.g. species of the family Lecythidaceae commonly typified by *Bertholletia excelsa* and *Barringtonia racemosa* [4], and *Theobroma cacao*, family Sterculiaceae [28]) or are adversely affected by excision and/or dehydration and liquid