

THE EFFECTS OF DESICCATION AND EXPOSURE TO CRYOGENIC TEMPERATURES OF EMBRYONIC AXES OF *Landolphia kirkii*

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Abstract

The present study reports on the effects of rapid dehydration, chemical cryoprotectants and various cooling rates on survival, assessed by the ability for both root and shoot development, of embryonic axes excised with a small portion of each cotyledon, from mature, recalcitrant seeds of *Landolphia kirkii*. All axes survived rapid (flash) drying to a water content of c. 0.28 g g⁻¹ (dry mass basis); however, the use of chemical cryoprotectants before flash drying was lethal. Rapid cooling rates were detrimental to axes flash-dried to 0.28 g g⁻¹, reducing survival to 10% and 0% after exposure to -196°C and -210°C, respectively. Ultrastructural examination revealed that compartmentation and loss of cellular integrity were associated with viability loss after rapid cooling to cryogenic temperatures, although lipid bodies retained their morphology. Hence, lipid crystallisation was not implicated in cell death following dehydration, exposure to cryogenic temperatures and subsequent thawing and rehydration. Cooling at 1°C min⁻¹ facilitated survival of 70% of axes with attached cotyledonary segments at 0.28 g g⁻¹ after exposure to -70°C, with 45% viability retention when further cooled at 15°C min⁻¹ to -180°C. However, no axes excised without attached cotyledonary segments produced shoots after cryogen exposure. The use of slow cooling rates is promising for cryopreservation of mature axes of *L. kirkii*, but only when excised with a portion of each cotyledon left attached.

Keywords: cooling rate, cryopreservation, dehydration, excision damage, *Landolphia kirkii*, lipid body, recalcitrant

INTRODUCTION

The recalcitrant seeds of *Landolphia kirkii* T.-Dyer (Apocynaceae) are desiccation- and chilling-sensitive (4, 11, 24, 34). If maintained at their original water content, seeds of *L. kirkii* will lose viability in approximately one month (24) as metabolic demands of the embryo exceed the endogenous seed tissue water. Cryostorage of the embryonic axes excised from *L. kirkii* seeds should offer a means of germplasm preservation. *Landolphia kirkii* is a scrambling shrub or woody climber, distributed along coastal and sandforest areas, mainly in the northern regions of KwaZulu-Natal, South Africa. The plant produces narrowly oval, large