Ngobese, N.Z., Sershen, Pammenter, N.W. and Berjak, P. (2010), *Seed Sci. & Technol.*, **38**, 704-716

Cryopreservation of the embryonic axes of *Phoenix reclinata*, a representative of the intermediate seed category

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(Accepted August 2010)

Summary

The responses of the embryonic axes of *Phoenix reclinata* to some of the pivotal procedures employed for cryopreservation were investigated, the aim being to develop a cryopreservation protocol for germplasm of this species. This was undertaken by subjecting excised embryonic axes to flash drying to achieve partial dehydration, and thereafter to cryogenic storage temperatures by employing slow (1°C min^{-1}, to -70°C and subsequently rapidly cooled) and rapid (hundreds of °C s^{-1}) cooling techniques, after which viability retention was monitored. The results showed that the embryonic axes of *P. reclinata* can withstand substantial dehydration in the short-term, allowing reductions in water content to as low as $\geq 0.05 \pm 0.03$ g g^{-1} (dry mass basis [dmb]) without any immediate detrimental effects on viability. Furthermore, these axes showed relatively good survival (≥ 80%) using both slow and rapid cooling rates, when cooled at optimum water contents (between 0.21 ± 0.12 and 0.25 ± 0.06 g g^{-1}). These findings indicate that cryopreservation offers a means for conservation of the germplasm of *P. reclinata*.

Introduction

Over the past five decades, storage of living cells at sub-zero temperatures, commonly referred to as cryopreservation, has been shown to be a viable method for conserving biological resources (Lane, 2004). However, the use of this technique in preserving germplasm has been limited because of the complexity in tissue characteristics, structure and cell-to-cell interactions (Muldew *et al.*, 2004). This has been a stumbling block in plant genetic resources conservation, since cryopreservation seems to be the only promising method for the conservation of germplasm of species for which seeds cannot be stored using conventional practices (Hong *et al.*, 1996; Berjak, 2000; Sershen *et al.*, 2007).

Conventional cryopreservation protocols generally include partial dehydration, the use of cryoprotective additives (CPA) and exposure of living biological material to ultra-low cooling temperatures (Muldew *et al.*, 2004; Panis and Lambardi, 2005). These measures are aimed at facilitating the reduction and subsequent interruption of metabolic function for cryogenic storage (often to the extreme temperature of liquid nitrogen [LN], *viz.* -196°C), for the preservation of genetic resources (Mycock *et al.*, 1995; Sakai, 2004).

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