A NOVEL MEANS FOR CRYOPRESERVATION OF GERMPLASM OF THE RECALCITRANT-SEEDED SPECIES, *Ekebergia capensis*

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Abstract

Cryopreserved zygotic embryonic axes offer the best means of genetic diversity conservation of recalcitrant-seeded species, but frequently shoots fail to develop following processing for, and after, cryostorage. The present work offers a means to overcome this, by generating adventitious shoots from seedling roots produced after axis cryopreservation. Embryonic axes of *Ekebergia capensis* were exposed to cryoprotectants, flash dried, and rapidly cooled in nitrogen slush. Cryoprotection was an essential step, with both glycerol and DMSO facilitating survival after cryogen exposure, but sucrose alone, or in combination with glycerol, was deleterious. Adventitious shoots were formed from seedling roots developed by axes germinated after cryogen exposure, after being subjected to intermittent flushing with a BAP-containing medium for 24 h in a temporary immersion system and subsequent culture on a semi-solid BAP-containing medium. After excision, a high proportion of the adventitious shoots produced roots *in vitro*, with most of these rooted plantlets being subsequently successfully acclimated.

Keywords: adventitious shoots; cryoprotection; plantlets; post-cryopreservation; recalcitrant seeds; temporary immersion.

INTRODUCTION

In contrast to orthodox seeds, which can be stored in the desiccated state at low temperature and relative humidity (RH), recalcitrant seeds remain hydrated and metabolically active, with those of most species losing viability upon slow removal of even a small proportion of cell water. Although rapid dehydration facilitates recalcitrant seed survival to considerably lower water contents than does slow drying (1,17), threshold levels for viability loss are too high to permit survival of exposure to cryogenic temperatures. With few exceptions, recalcitrant seeds are not only too wet, but also too large, to be cryostored. Because of their desiccation sensitivity – and because their ongoing metabolism moves inexorably into germination – storing recalcitrant seeds is possible only in the hydrated state, and this is strictly a short- to medium-term option (1). Zygotic embryonic axes excised from such desiccation-sensitive seeds offer the ideal explants in terms of cryo-conservation of genetic diversity of the species concerned. However, a prevailing problem with tropical/sub-tropical woody species, is that shoot production by excised axes – whether cryopreserved or