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CRYOPRESERVATION, ENCAPSULATION AND PROMOTION OF SHOOT PRODUCTION OF EMBRYONIC AXES OF A RECALCITRANT SPECIES *Ekebergia capensis*, Sparrm.

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Abstract

A study on zygotic axes of the recalcitrant seeds of *Ekebergia capensis* compared two cryopreservation methods, partial desiccation, and encapsulation-dehydration, and also investigated a method to promote shoot production. High (80%) survival (assessed as root production) was obtained after direct immersion into liquid nitrogen of axes rapidly dehydrated by flash drying for 20 min to a water concentration of *c.* 0.4 g g^{-1}. In contrast, no survival at all was obtained of axes that were first encapsulated, then desiccated for three hours to the same water concentration as those fast-dried, and then placed in a cryovial and immersed in liquid nitrogen. Axes encapsulated after cryopreservation germinated both *in vitro* and in soil, and could be stored at room temperatures for several weeks while maintaining germinability, thus producing synseeds capable of distribution. However, shoot production after cryopreservation was seldom observed. The inclusion of the plant growth regulator, N^6-benzyl adenine (BA) in the MS-based recovery medium promoted vigorous multiple shoot formation. Microscopical examination of embryos of *E. capensis* revealed that the cotyledonary insertions were contiguous with the shoot apex, leading to the conclusion that injury to, and ultimate necrosis of, the apical meristem following severing of these connections was a primary cause of the observed lack of, or poor, shoot development in excised axes (whether cryopreserved or not). The study demonstrated that it may be possible to resolve two of the problems facing attempts at cryopreservation of axes of recalcitrant seeds; lack of shoot production and difficulty of distribution of cryopreserved material for re-introduction.

Keywords: cooling rate; cryopreservation; encapsulation; BA (N^6-benzyladenine); synseed; recalcitrant seed.

INTRODUCTION

Cryopreservation is a technique that should overcome the problems of genetic instability encountered during long-term conservation of genetic resources. The technique has the potential to facilitate preservation of the germplasm of recalcitrant seed-producing