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Developmental status is a critical factor in the selection of excised recalcitrant axes as explants for cryopreservation: a study on *Trichilia dregeana* Sond.

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Abstract

As a consequence of previous lack of success in cryostoring axes excised from newly shed seeds of *Trichilia dregeana*, the effects of the mode of axis excision on seedling production were investigated. Although vigorous root production occurred, no shoots were produced when the cotyledons were severed as closely as possible to the axis surface (explant-type 0). In contrast, shoot production was increasingly facilitated when small to larger segments of cotyledonary tissue were left attached to the axes (explant-types 1, 2 and 4), which did not compromise axis drying rate. However, root growth of explant-types 1, 2 and 4 was negatively affected, probably by leakage into the medium of an inhibitory or toxic substance(s) from the cut surfaces of the cotyledonary tissue. Microscopical examination revealed that the cotyledons were sessile, and their insertions were contiguous with the shoot apex in axes from newly shed seeds, leading to the suggestion that failure of shoot production by type 0 explants *in vitro* was the direct consequence of the proximity of the wound sites to the apical meristem. When seeds were stored hydrated for 6 months, the shoot apex had elongated, positioning the apical meristem some distance from the top of the cotyledonary insertions. In contrast to axes excised as type 0 explants from newly shed seeds, the equivalent explants from the stored seeds rapidly formed shoots and leaves *in vitro*. This indicates that the developmental status of axes, when excised, dictates failure or success in their further development *in vitro*, and that this aspect needs to be resolved before any further manipulations for cryostorage are attempted.

Keywords: embryonic axis, recalcitrant seed, seed development, seed storage, shoot production, *Trichilia dregeana*

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Introduction

Recalcitrant seeds are continuously sensitive to dehydration, the degree varying with the developmental status and the rate at which water is lost (Pammenter and Berjak, 1999; Berjak and Pammenter, 2001, 2004). Seeds falling into the intermediate category, as defined by Hong and Ellis (1996), and considered to occupy different positions on the continuum between true recalcitrance and orthodoxy (Pammenter and Berjak, 1999; Berjak and Pammenter, 2001, 2004) are relatively desiccation-tolerant, but do not survive the extent of water loss naturally withstood by orthodox types. While short- to medium-term storage of recalcitrant seeds is generally optimized by their maintenance in the hydrated condition after treatment to eliminate or curtail associated microorganisms (Calistru *et al.*, 2000; Anguelova-Merhar *et al.*, 2003; Berjak and Pammenter, 2004), intermediate types should retain viability at the lowest water concentration tolerated without immediate deleterious effects. As recalcitrant and intermediate seeds of many species are chilling-sensitive, low-temperature storage is often precluded. Despite optimization of seed water concentration and storage temperature, these approaches to non-orthodox seed storage do not facilitate long-term maintenance of vigour and viability.