



## Calcium, with magnesium, is essential for normal seedling development from partially dehydrated recalcitrant axes: a study on *Trichilia dregeana* Sond.

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### Abstract

Assessment of the rehydration procedure has been consistently overlooked in evaluation of factors contributing to successful cryostorage of partially dehydrated, embryonic axes excised from recalcitrant seeds. Conventional rehydration of *Trichilia dregeana* (Sond.) axes in distilled water resulted in the lack of root pole gravitropism after culture on medium *in vitro*. In comparison, a strong gravitropic response was observed in axes that had not been dehydrated, and by those rehydrated in a solution containing 1  $\mu\text{M}$   $\text{CaCl}_2$  and 1 mM  $\text{MgCl}_2$ . However, no marked loss of either cation from the tissue could be detected, whether axes were rehydrated in distilled water or the  $\text{Ca}^{2+}/\text{Mg}^{2+}$  solution. Starch-packed statoliths differentiated rapidly in both non-dehydrated axes and those rehydrated in the divalent cation solution, but these organelles failed to develop or accumulate much starch following axis rehydration in water, as was the case for the amyloplasts of root cells generally. After rehydration in the  $\text{Ca}^{2+}/\text{Mg}^{2+}$  solution and 48 h in culture, axis root-cap columella cells accumulated  $\text{Ca}^{2+}$ , whereas axes rehydrated in water or solutions containing either  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  alone did not take up  $\text{Ca}^{2+}$ . Rehydration with the  $\text{Ca}^{2+}/\text{Mg}^{2+}$  solution also altered distribution of the actin component of the cytoskeleton. When rehydrated in the divalent cation solution, actin was associated with the nucleus and with the statoliths, which were located distally in statocytes of axes. In contrast, actin was largely confined to the

perinuclear area in root-cap columella cells of the agravitropic, water-rehydrated axes. The present results indicate a definitive primary role for  $\text{Ca}^{2+}$  with  $\text{Mg}^{2+}$  in graviperception, via starch metabolism, and in the determination of statolith morphology, which appears to be linked with maintenance of the actin component of the cytoskeleton in root-cap statocytes.

**Keywords:** amyloplast, calcium, cryostorage, cytoskeleton, embryonic axes, graviperception, magnesium, rehydration, starch metabolism, statocytes, statoliths, *Trichilia dregeana*

### Introduction

Cryostorage of non-orthodox, desiccation-sensitive plant genetic resources, as represented by plants that produce intermediate and recalcitrant seeds, usually involves conservation of excised, partially dehydrated zygotic embryonic axes in liquid nitrogen. To produce normal plants after cryopreservation of embryonic axes (or any other planting stocks), a carefully defined sequence of manipulations is required before, and after, introduction of the axes into liquid nitrogen, all of which have the potential to induce abnormalities (Berjak *et al.*, 1999). These manipulations include: axis excision; surface-sterilization at this point or later; partial dehydration (for which extent and rate of water loss are critical features); cooling rate when axes are introduced into the cryogen; thawing parameters after cryostorage; rehydration that may be simultaneous with, or immediately follows, thawing; *in vitro* conditions promoting ongoing axis germination and seedling formation; and hardening-off and planting in soil.

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