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The influence of rehydration technique on the response of recalcitrant seed embryos to desiccation

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Abstract

The concept of 'imbibitional damage' arose when it was observed that considerable leakage of cell contents could occur when dry seed or pollen tissues are plunged directly into water. It is now common practice to imbibe dehydrated tissue slowly, to permit the re-establishment of functional membranes, prior to placing the tissue into liquid water. However, this argument may not hold if the tissue of interest is inherently desiccation-sensitive. Slow drying of desiccation-sensitive (recalcitrant) seeds or excised embryonic axes results in damage at high water contents, because it permits time for aqueous-based deleterious processes to occur. The same argument may apply if partially dried material is re-imbibed slowly, as this technique will also expose the tissue to intermediate water contents for protracted periods. This hypothesis was tested using embryos or axes from seeds of three recalcitrant species (*Artocarpus heterophyllus*, *Podocarpus henkelii* and *Ekebergia capensis*). Excised material was rapidly dried to water contents within the range over which viability is lost during drying, and re-imbibed either rapidly, by plunging directly into water, or slowly, by placing the material on damp filter paper or exposing it to a saturated atmosphere for several hours. Although details of the response differed among species and developmental stage, in all cases direct re-imbibition in water resulted in higher (or similar, but never lower) survival than either of the slow rehydration techniques.

Keywords: dehydration, desiccation-sensitivity recalcitrant, rehydration, seeds

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Introduction

When dry desiccation-tolerant tissue is imbibed in water, there is a transient leakage of solutes (discussed by Hoekstra *et al.*, 1999). If the tissue is very dry, or if imbibition occurs at low temperatures, leakage can be extensive, reducing vigour (Pollock, 1969; Hobbs and Obendorf, 1972; Bramlage *et al.*, 1978). These observations led to the concept of 'imbibitional damage', which can be reduced by pre-humidification in water vapour-saturated air prior to imbibition (e.g. Hobbs and Obendorf, 1972; Bramlage *et al.*, 1978; Hoekstra and van der Wal, 1988), or by slow rehydration (Vertucci, 1989 for seeds; Kosanke *et al.*, 1992 for dried bacteria), and it is now common practice to pre-humidify dry material prior to imbibition.

Recalcitrant seeds are damaged at water contents far in excess of those at which imbibitional damage occurs, and there is little evidence to suggest that such damage is important in desiccation-sensitive material. None the less, on the basis of the 'safety-first' principle, such pre-humidification has been used in some studies (e.g. Berjak *et al.*, 1992, 1993; Leprince *et al.*, 1998; Pammenter *et al.*, 1998). An assumption implicit in this 'safety-first' principle is that slow rehydration, if not beneficial, at least is not damaging to the partially dehydrated tissue. However, this may not be true. It is well known that the rate of drying can affect the water content to which recalcitrant seeds or excised embryonic axes can be dried before loss of viability occurs, with slow drying leading to damage at higher water contents (Farrant *et al.*, 1985; Normah *et al.*, 1986; Pritchard, 1991; reviewed by Pammenter and Berjak, 1999). It is possible that a similar phenomenon may occur on rehydration; i.e. slow rehydration could lead to the further accumulation of damage. The objective of the study