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The Effects of Two Drying Rates on the Desiccation Tolerance of Embryonic Axes of Recalcitrant Jackfruit (*Artocarpus heterophyllus* Lamk.) Seeds

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This study compared the survival, electrolyte leakage and ultrastructural characteristics of embryonic axes of recalcitrant jackfruit dried rapidly ($\leqslant$90 min) or slowly (2–3 d). Axes dried slowly showed decreased viability at higher water content than those dried rapidly; this was mirrored by an increase in electrolyte leakage at approx. 0·8 and 0·4 g H_2O g^{-1} dry mass, respectively. Rapid drying conferred relatively greater tolerance to dehydration, as attested by the 100 % survival attained at approx. 0·4 g g^{-1} in contrast to the total mortality of axes dried slowly to this water content. Partially hydrated axes were processed for microscopy using freeze-substitution to prevent rehydration artefacts. Radicles of axes dehydrated to 0·7 g g^{-1} were examined microscopically to assess their cellular morphology and appearance of membranes following rapid or slow drying. Measurements showed that drying rate affected the distribution of water within axes, which could be relevant to the extent of stress experienced by germinative cells. Membrane breakdown was not observed in cells dehydrated either rapidly or slowly to 0·7 g g^{-1}. Endoplasmic reticulum profiles were prominent in dehydrated cells. Autophagy was observed in axes of both treatments. The area occupied by vacuoles increased significantly only following rehydration, and was similar in axes dried rapidly or slowly. Desiccation damage became evident on rehydration, and was reversible following rapid drying but was more severe in axes dried slowly. Prolonged exposure to partial hydration may contribute to the greater sensitivity of vacuoles to damage during rehydration.

Key words: *Artocarpus heterophyllus* Lamk. (jackfruit), autophagy, cryopreservation, desiccation-damage, drying rates, electrolyte leakage, endoplasmic reticulum, freeze substitution, recalcitrance.

INTRODUCTION

The development of strategies for the long-term storage of desiccation-sensitive germplasm remains a challenge for those involved in the conservation of endangered and commercially important species producing recalcitrant seeds. Although cryopreservation is the most likely solution to this problem, success is challenged by the fact that recalcitrant seeds are large and are shed at high water content. Lethal freezing damage invariably occurs if hydrated seeds are exposed to liquid nitrogen, while drying to water contents where ice does not form usually leads to desiccation damage and loss of viability (King and Roberts, 1980). Achieving harmless dehydration of recalcitrant germplasm to water contents that allow survival following exposure to cryogenic temperatures is critical to the establishment of successful cryopreservation procedures (King and Roberts, 1980; Becwar *et al*., 1983; Pritchard and Prendergast, 1986; Pence, 1992, 1995; Wesley-Smith *et al*., 1992; Chandel *et al*., 1995).

The lower limit of desiccation tolerance varies among species, and even within species depending upon the developmental status (Farrant *et al*., 1986, 1989; Hong and Ellis, 1990; Berjak *et al*., 1992, 1993; Finch-Savage, 1992; Tompsett and Pritchard, 1993). Evidence suggests that rapid drying of whole seeds allows lower water contents to be attained whilst still retaining viability (Farrant *et al*., 1986; Berjak *et al*., 1989, 1990; Pritchard, 1991; Pammenter *et al*., 1998). Physical constraints limit the rate of dehydration of whole seeds, which leads to the loss of viability at relatively high water contents (Farrant *et al*., 1986). This obstacle can be overcome by exposing excised embryonic axes to an airstream (flash drying; Berjak *et al*., 1989, 1990). This drying technique has facilitated dehydration of axes to water contents previously unattained with whole seeds yet with minimal loss of viability (Normah *et al*., 1986; Pritchard and Prendergast, 1986; Berjak *et al*., 1989; Pammenter *et al*., 1991; Pritchard, 1991; Pritchard and Manger, 1998). In spite of this, a lower limit to desiccation tolerance still persists and exposure to such low water contents can result in desiccation damage (reviewed by Vertucci and Farrant, 1995; Pammenter and Berjak, 1999; Walters *et al*., 2001*a*).

The mechanisms by which rapid drying allows lower water contents to be tolerated in recalcitrant material have not yet been fully elucidated, but evidence indicates that the duration of the benefit conferred is short (Pammenter *et al*., 1998; Walters *et al*., 2001*b*; reviewed by Vertucci and Farrant, 1995; Walters *et al*., 2001*a*). It has been suggested that normal functions may be perturbed at water contents

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