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THE INFLUENCE OF WATER CONTENT, COOLING AND WARMING RATE UPON SURVIVAL OF EMBRYONIC AXES OF *Poncirus trifoliata* (L.)

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Abstract

The present study investigated the relative contributions of water content and non-equilibrium cooling and warming rates to the survival of cryopreserved axes of recalcitrant *P. trifoliata* seeds. Reducing water contents from 1.7 and 0.26 $gH_2O\ g^{-1}$ dry mass ($g\ g^{-1}$) is believed to increase cytoplasmic viscosity. Cooling to -196°C was done at rates averaging between 0.17 and 1300°C s^{-1}, and warming at 600 or 1.35°C s^{-1}. Survival was assessed after 4 weeks *in vitro*. Rapid warming resulted in higher survival and normal development of axes at all water contents. The effects of cooling rate were dependent on the water content of axes. Cooling rates resulting in >70% normal development ranged between 0.17 and about 1300°C s^{-1} for axes at a water content of 0.26 $g\ g^{-1}$, narrowing with increasing hydration to an apparent optimum at about 686°C s^{-1} in axes at 0.8 $g\ g^{-1}$. At 1.7 $g\ g^{-1}$, axes cooled at 0.17°C s^{-1} yielded nearly 40% normal development, whereas faster cooling was deleterious. Results are interpreted in the context of the effect of water content on cytoplasmic viscosity and the rate of intracellular ice formation. At low water contents, the high intracellular viscosity slows ice crystallization making survival independent of cooling rate. At higher water contents, the reduced viscosity requires faster cooling to prevent ice crystal damage. The ability to cool rapidly with increasing hydration is balanced with an increasing limitation to dissipate heat fast enough to prevent severe damage.

Keywords: embryonic axes, rapid cooling, cytoplasmic viscosity, *Poncirus trifoliata*, warming, recalcitrant seed, water content, cryopreservation

INTRODUCTION

Water content influences cytoplasmic viscosity and, therefore, the mobility of water within cells (3, 12, 11, 42, 51). When seed embryonic axes are exposed to rapid (non-equilibrium) cooling, the mobility of water and the cooling rate required to prevent the formation of lethal ice crystals are interlinked (17, 32). The intracellular viscosity attained by drying to *c.* 0.25 $g\ g^{-1}$ ($\Psi \approx$ -12 MPa) is sufficient to limit ice formation during relatively slow cooling, making survival of axes independent of the time of exposure to temperatures that promote crystallization (39, 40, 48). While axes of many species tolerate this extent of drying and survive cryogenic exposure, those of more desiccation-sensitive species die at relatively higher water contents, which makes their cryopreservation challenging. The greater mobility of water with increasing hydration demands that axes be cooled correspondingly faster if mechanical injury by crystallisation is to be avoided (48, 47). Eventually a threshold is reached, which is set by the difficulty in dissipating the greater heat load of hydrated specimens