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Why is intracellular ice lethal? A microscopical study showing evidence of programmed cell death in cryo-exposed embryonic axes of recalcitrant seeds of *Acer saccharinum*

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Background and Aims Conservation of the genetic diversity afforded by recalcitrant seeds is achieved by cryopreservation, in which excised embryonic axes (or, where possible, embryos) are treated and stored at temperatures lower than −180 °C using liquid nitrogen. It has previously been shown that intracellular ice forms in rapidly cooled embryonic axes of *Acer saccharinum* (silver maple) but this is not necessarily lethal when ice crystals are small. This study seeks to understand the nature and extent of damage from intracellular ice, and the course of recovery and regrowth in surviving tissues.

Methods Embryonic axes of *A. saccharinum*, not subjected to dehydration or cryoprotection treatments (water content was 1·9 g H_2O g^{-1} dry mass), were cooled to liquid nitrogen temperatures using two methods: plunging into nitrogen slush to achieve a cooling rate of 97 °C s^{-1} or programmed cooling at 3·3 °C s^{-1}. Samples were thawed rapidly (177 °C s^{-1}) and cell structure was examined microscopically immediately, and at intervals up to 72 h *in vitro*. Survival was assessed after 4 weeks *in vitro*. Axes were processed conventionally for optical microscopy and ultrastructural examination.

Key Results Immediately following thaw after cryogenic exposure, cells from axes did not show signs of damage at an ultrastructural level. Signs that cells had been damaged were apparent after several hours of *in vitro* culture and appeared as autophagic decomposition. In surviving tissues, dead cells were sloughed off and pockets of living cells were the origin of regrowth. In roots, regrowth occurred from the ground meristem and procambium, not the distal meristem, which became lethally damaged. Regrowth of shoots occurred from isolated pockets of surviving cells of peripheral and pith meristems. The size of these pockets may determine the possibility for, the extent of and the vigour of regrowth.

Conclusions Autophagic degradation and ultimately autolysis of cells following cryo-exposure and formation of small (0·2–0·4 μm) intracellular ice crystals challenges current ideas that ice causes immediate physical damage to cells. Instead, freezing stress may induce a signal for programmed cell death (PCD). Cells that form more ice crystals during cooling have faster PCD responses.