Intracellular Ice and Cell Survival in Cryo-exposed Embryonic Axes of Recalcitrant Seeds of *Acer saccharinum* L.: An Ultrastructural Study of Factors Affecting Cell and Ice Structures.

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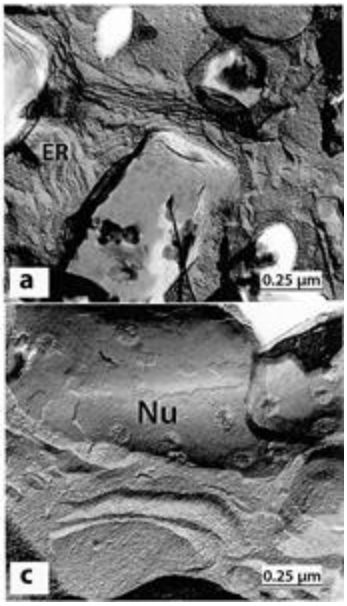
INTERPRETIVE SUMMARY:

Recalcitrant seeds do not survive drying and so cannot be preserved using conventional storage conditions in the freezer. Cryogenic technologies are required to preserve genetic diversity in species producing recalcitrant seeds such as oaks, some maples and many tropical fruits. The key to cryopreservation is finding the right balance between partial drying and extremely rapid cooling to avoid ice formation. The process creates aqueous glasses that stabilize cellular structures. This study demonstrates that tiny ice crystals form when hydrated embryonic axes are cooled very rapidly -- at about 100°C per sec. Even with some small ice crystals present, cell structure is maintained. The study shows stringencies and flexibilities required to effect cryopreservation protocols for recalcitrant seeds.

WHY THIS PAPER IS COOL:

We wanted to cool the seeds as fast as possible and so we invented different ways to inject embryonic axes into liquid nitrogen; one method used a spring-loaded desoldering pump and we measured cooling rates as fast as 300°C per sec. Once cooled, we could examine whether the cells blew up or how much ice formed using electron microscopy techniques that gave resolution at 0.01 microns. We found that we could control the size of ice crystals depending on the amount of water in the cells and how fast we could cool them. When axes survived, the ice crystal diameter was less than 0.4 microns, which is about ten times smaller than ice crystals in smooth frozen desserts, like ice cream.

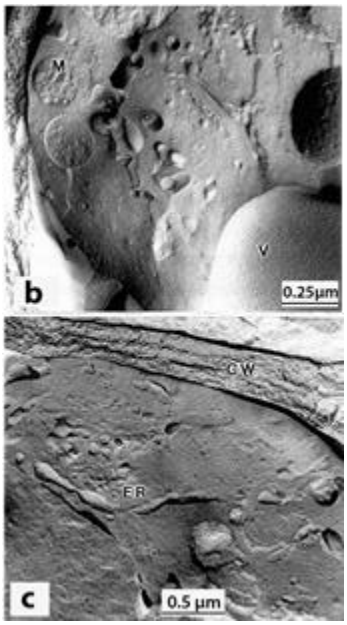
Embryonic axis of silver maple



These photos show freeze fracture electron micrographs of the inside of some cells in the root that were cooled at 3°C per sec. These roots survived liquid nitrogen and grew normally.The endoplasmic reticulum (ER) in the top photo is compressed by ice crystals.

The lower picture shows the nucleus (Nu) of a cell which maintained good structure despite botchy patches on the membrane surface, indicating some distortions during freezing.

These photos are freeze fracture electron micrographs of root cells that were slightly dried and cooled at about 150°C per sec. This treatment resulted in ice crystals too small to detect (< 0.01 microns) and so the preservation of cellular structure is excellent. Here, you can see the cell vacuole (V) and mitochondria (M), endoplasmic reticulum (ER) and cell wall (CW).



WHAT WE LEARNED:

It is nearly impossible to prevent water from freezing when working with tissues as large as maple embryonic axes that contained a lot of water. But, with a little drying and very fast cooling, ice crystals could be kept so small that we could not detect them with our techniques. When the ice crystals were small, cells stayed intact. Our next question is what happens when we warm these cells?