ability of ZRA to inhibit ice recrystallization and to reduce ice crystal growth rate imply on its tremendous potential usages in the field of cryopreservation and other biological and engineering fields.

Supported by IIF, ERC and IRC.

Conflict of interest: None declared.
E-mail address: braslavslagi@hi.huji.ac.il (I. Braslavsky)

http://dx.doi.org/10.1016/j.cryobiol.2012.07.049

49. Interactions between water and triacylglycerols may explain faster aging rates in stored germplasm at low temperatures. Aline Schneider Teixeira 1, Daniel Ballesteros 2, Antonio Diego Molina-Garcia 1, Christina Walters 2, 1 ICTAN (CSIC), Jose Antonio Novais 10, 28040 Madrid, Spain, 2 INRA, UMR 782 Génie et Microbiologie des Procédés Crystallization kinetics. Freezes in seeds increases with storage duration in a manner consistent with TAG dependent on water content and occurs over several days. The amount of water that assessed over a 3 week period. We report that TAG crystallization in seeds is inde-

fixatives using low temperature scanning electron microscopy (cryo-SEM) per-

/C0

tered numerous seed and fern spore species that age faster when stored in the free-

zer near –20°C compared to storage at higher or lower temperatures. Seeds exhibiting this temperature anomaly are considered to be intermediate between orthodox and recalcitrant physiologies. Intermediate traits are often associated with high proportions of saturated fatty acids within the triacylglycerols (TAG), accumulated into cells as food reserves. TAG high in saturated fatty acids crystall-

ize and melt within the temperature range of the observed longevity anomalies and we have hypothesized a link between TAG, seed change and increased aging to germplasm cells. Damage is exacerbated as water content within stored seeds and fern spores increases from near 5% to about 12%. These observations have led us to hypothesize that damage results from an interaction between TAG, water, temperature and time. The purpose of this research is to explore the extent and significance of possible interacting factors on the viability of stored germplasm. Our work begins with characterizing the kinetics of TAG and water phase changes in peanut (Arachis hypogaea) and papaya (Carica papaya) seeds equilibrated to different water contents and stored at temperatures between –5 and –80°C. Water and TAG phase was measured using a Perkin Elmer Differential Scanning Calorimeter. Cytoplasm ultra-structure was visualized without chemical fixatives using low temperature scanning electron microscopy (cryo-SEM) per-

formed with a Zeiss DSM 960 scanning microscope equipped with a Cryotrans CT–1500 cold plate (Oxford, UK). The time dependency of phase changes was assessed over a 3 week period. We report that TAG crystallization in seeds is inde-

pendent of water content and occurs over several days. The amount of water that freezes in seeds increases with storage duration in a manner consistent with TAG crystallization kinetics.

Conflict of interest: None declared.
Source of funding: None declared.
E-mail address: spassot@grignon.inra.fr (S. Passot)

http://dx.doi.org/10.1016/j.cryobiol.2012.07.051

Anthony J. Reardon received the John K. Critser Travel Award

51. A comparative assessment of mitochondria and membrane cryobiological responses of H. UVEC to low temperatures. Anthony J. Reardon 1, Janet A. W. Elliott 2, Locksley E. McGann 3, 1 Departments of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada, 2 Departments of Laboratory Chemical and Materials Engineering, University of Alberta, Edmonton, Alberta, Canada

The extent of cellular cryoinjury incurred during cryopreservation protocols is traditionally assessed with membrane integrity assays, often used as an upper limit in determining viability. However, exposure of cells to subzero temperatures and conditions may lead to changes in various aspects of cellular structure and function that would not be considered in studies that rely on membrane integrity assess-

ments alone. Slower cooling rates in particular have been shown to adversely affect the metabolism (McGann et al., 1988; and Sherman, 1972) and structural properties (Tchir et al., 2010) of mitochondria in addition to the occurrence of damage to the plasma membrane during freezing and thawing stress. The objective of the study was to use a mitochondrial assay to investigate a secondary aspect of cell response to directly compare with a traditional assessment of membrane integrity under identical con-

ditions. An interrupted slow cooling method was used to examine the occurrence of damage from slow cooling to intermediate subzero experimental temperatures, and damage during rapid cooling when plunged into liquid nitrogen. Human umbilical vein endothelial cells (H.UVECs) were cooled at 0.2°C/min to temperatures between –3°C and –40°C in the absence of cryoprotectant. Samples were then either thawed directly in a 37°C water bath or plunged into liquid nitrogen before thawing. The membrane integrity of cells was determined using a combination of the nuclear fluorescent dyes Syto13 and ethidium bromide, and mitochondrial polariza-

tion was indicated with the cationic carbocyanine dye JC-1. The number of cells determined to be membrane intact and the number of cells containing polarized mitochondria decreased with decreasing temperature in directly-thawed samples. Depolarized mitochondria was found in 50% of cells at –15°C, whereas 50% of cells were membrane compromised at a lower temperature (~30°C). H.UVEC plunged into liquid nitrogen from experimental temperatures showed an increase in the number of membrane intact cells with decreasing experimental temperature to a maximum of 40% at ~20°C, but <5% of these cells contained polarized mitochondria. Directly thawed samples showed a greater proportion of cells with depolarized mitochondria at higher subzero temperatures than cells with membrane damage, indicating that subcellular changes occur in cells under conditions that do not com-

promise the integrity of the plasma membrane. At some experimental tempera-

tures, plunged H.UVECs showed significant membrane integrity, immediately post-

thaw, but few of these membrane intact cells contained polarized mitochondria. This demonstrates that damage to organelles and the plasma membrane may occur