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Cryopreservation - emphasis on long-term security of crop germplasm and recalcitrant seeds

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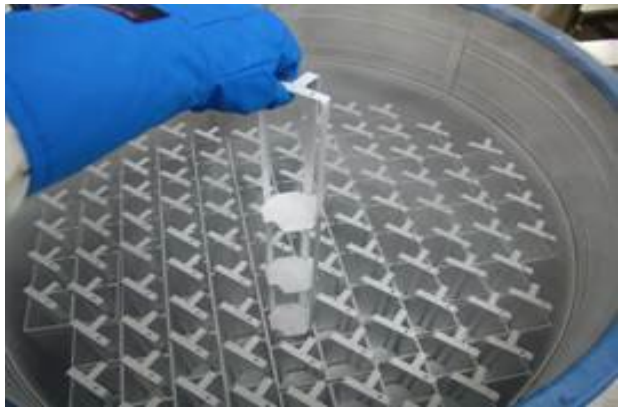


ISTA 100 YEARS
A CENTURY OF PROGRESS IN SEED QUALITY ASSURANCE

Importance of seed conservation

- Protecting food plant biodiversity
- Counter for the loss of diversity in human diets and in ecosystems
- To conserve and promote biodiversity for food and nutrition
- Insurance policy againt extinction
- Readily available source of material for research, re-introduction

Why cryopreserve?

“Orthodox” (desiccation-tolerant)

- seeds tolerate desiccation to low (c. 5%) moisture contents
- seed longevity increases with decreasing moisture content and lower temperature

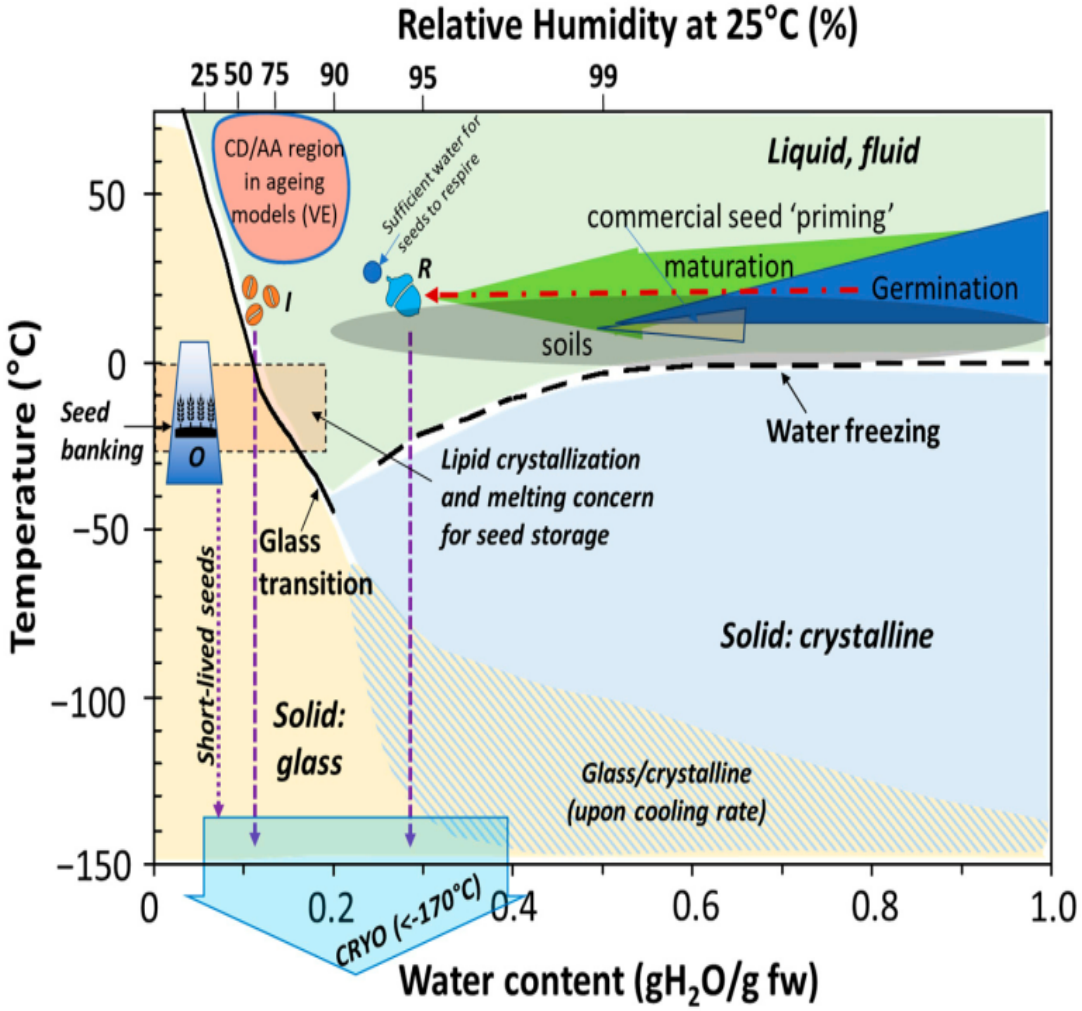
“Recalcitrant” (desiccation-sensitive)

- seeds killed by desiccation to comparatively high (20 – 25%) moisture contents
- storage at ambient is only possible for short periods (months)

“Intermediate” seeds

- tolerant to desiccation to a considerable extent
- dry seeds are more sensitive to storage at 10 °C temperature or below.

Phase diagram of seed physical states and storage challenges for the different seed storage behaviours



Problems with oily seed storage

Planta (2003) 217: 699–708
DOI 10.1007/s00425-003-1036-1

ORIGINAL ARTICLE

Jennifer Crane · Annette L. Miller
J. William Van Roekel · Christina Walters

Triacylglycerols determine the unusual storage physiology of *Cuphea* seed

Seeds behaving badly: Conservation of rainforest species

Australasian Plant Conservation: Journal of the Australian Network for Plant Conservation
Volume 21 Issue 3 (Dec 2012 - Feb 2013)

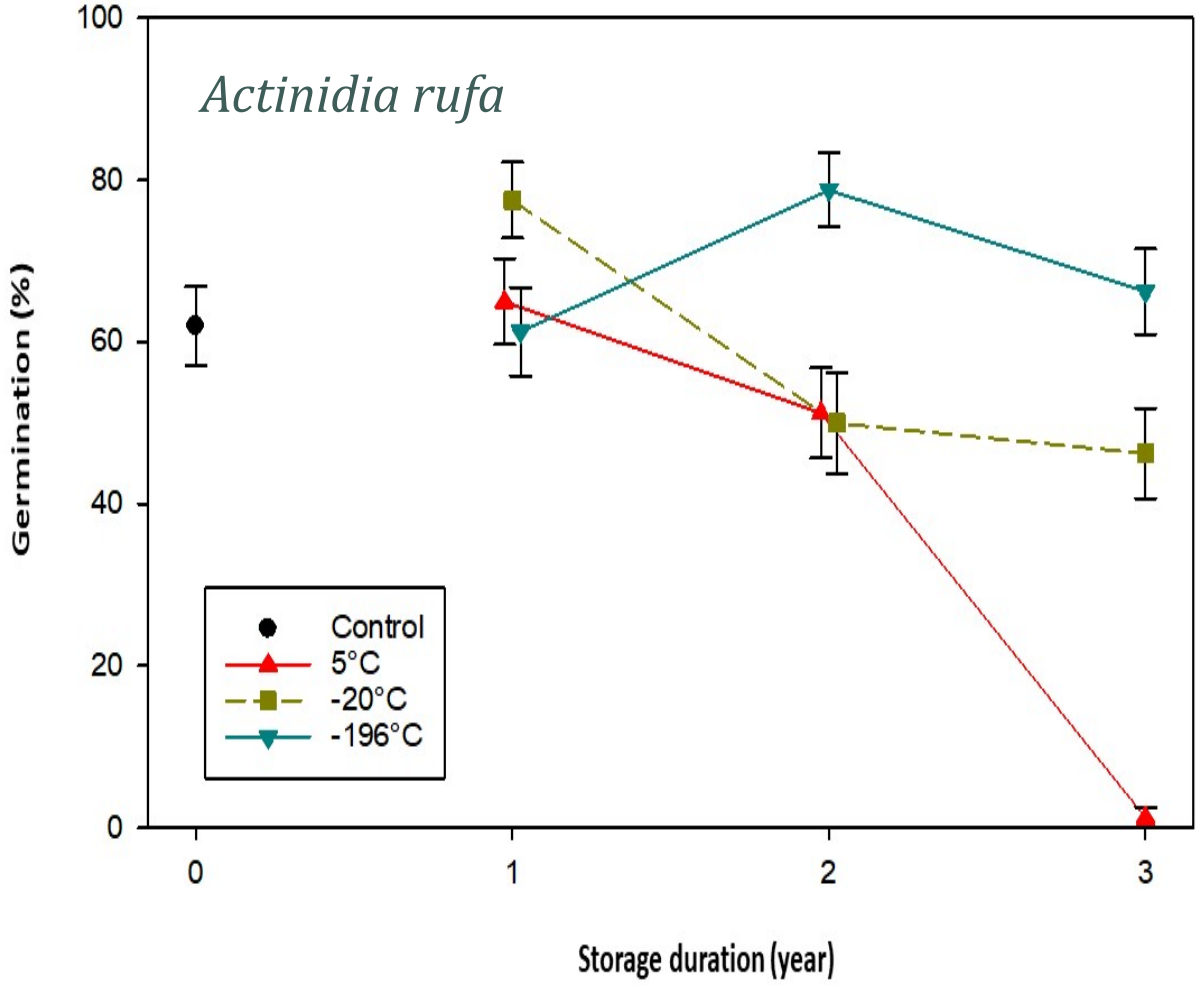
Errington, Graeme[1]; Cuneo, Peter[2]; Offord, Catherine A[3]

Factors that may influence oily seed storage

- Seed lipid content
- Fatty acid profile
- Lipid thermal behaviour
- Lipid body size

Kiwifruit (*Actinidia* sp) seed storage

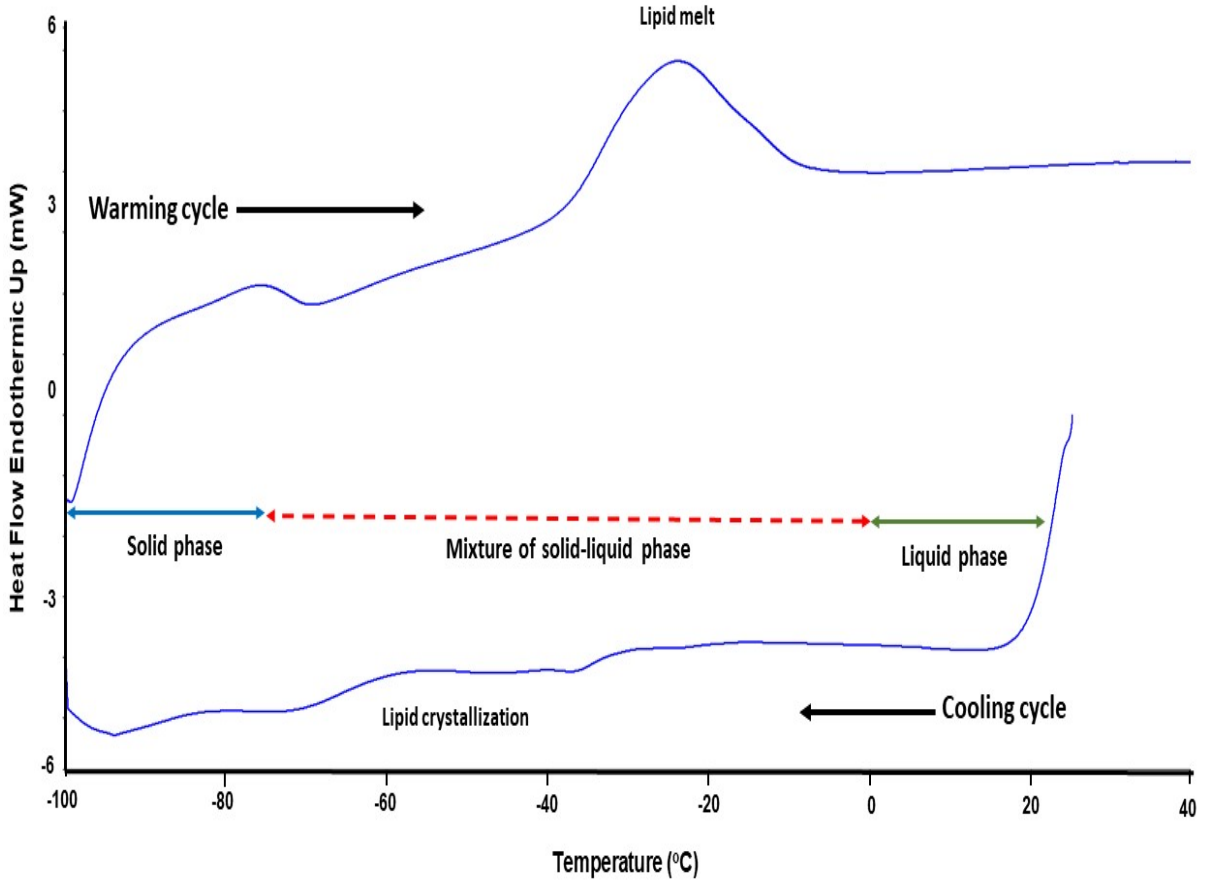
- Kiwifruit seeds - desiccation tolerant and should be bankable at −20°C.
- However, our previous study on seed storage of seven species at different temperatures for three years showed declines in seed germination at 5°C and −20°C.



Nadarajan et al 2024. Manuscript in press in Acta Horticulturae

Kiwifruit seed lipid thermal fingerprinting

- Kiwifruit seeds have high oil content (c. 30%)
- DSC thermal analysis revealed that their lipids are in transitional phase (mixture of liquid and solid phase) at −20°C
- Seed lipid thermal fingerprints for rapidly identifying potentially short-lived oily seeds under dry-cold storage environments – can be used as a biophysical marker



Differential scanning calorimetry (DSC) cooling and warming thermograms for *Actinidia rufa* seeds

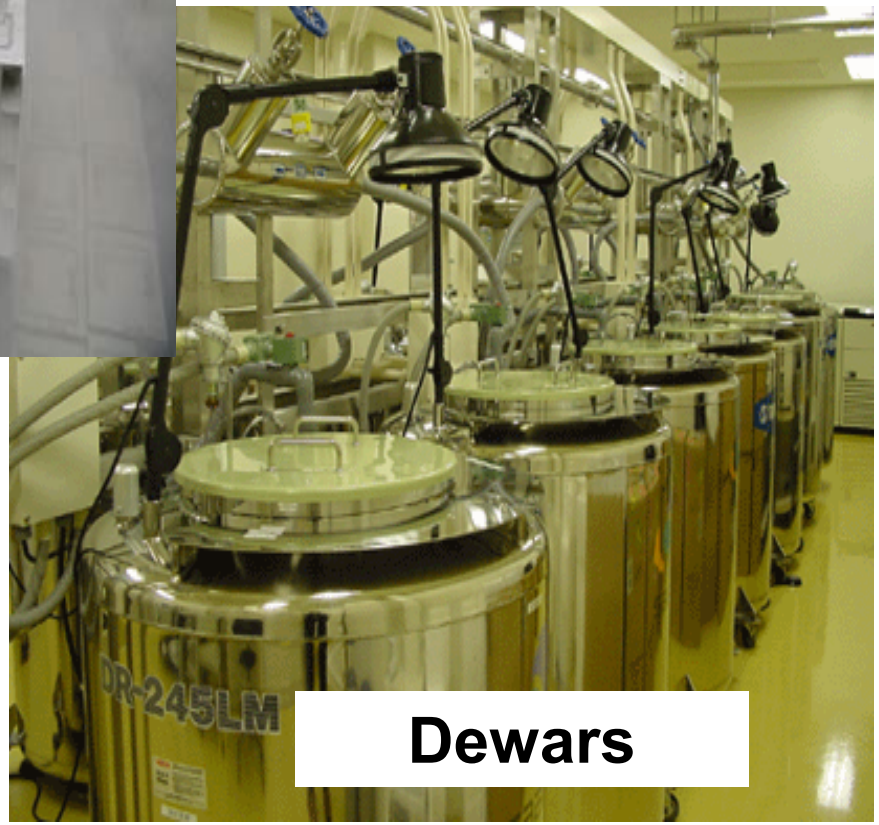
Nadarajan et al 2024. Manuscript in press in Acta Horticulturae

What is cryopreservation?

- Storage at below about -130 °C (cryogenic temperature) reduces deterioration rate as:
  - lack of liquid water
  - extremely slow diffusion
  - low molecular energies
  - infrequent chemical reactions

- Potential for ‘indefinite’ preservation

- Only option for preservation of desiccation sensitive seeds species



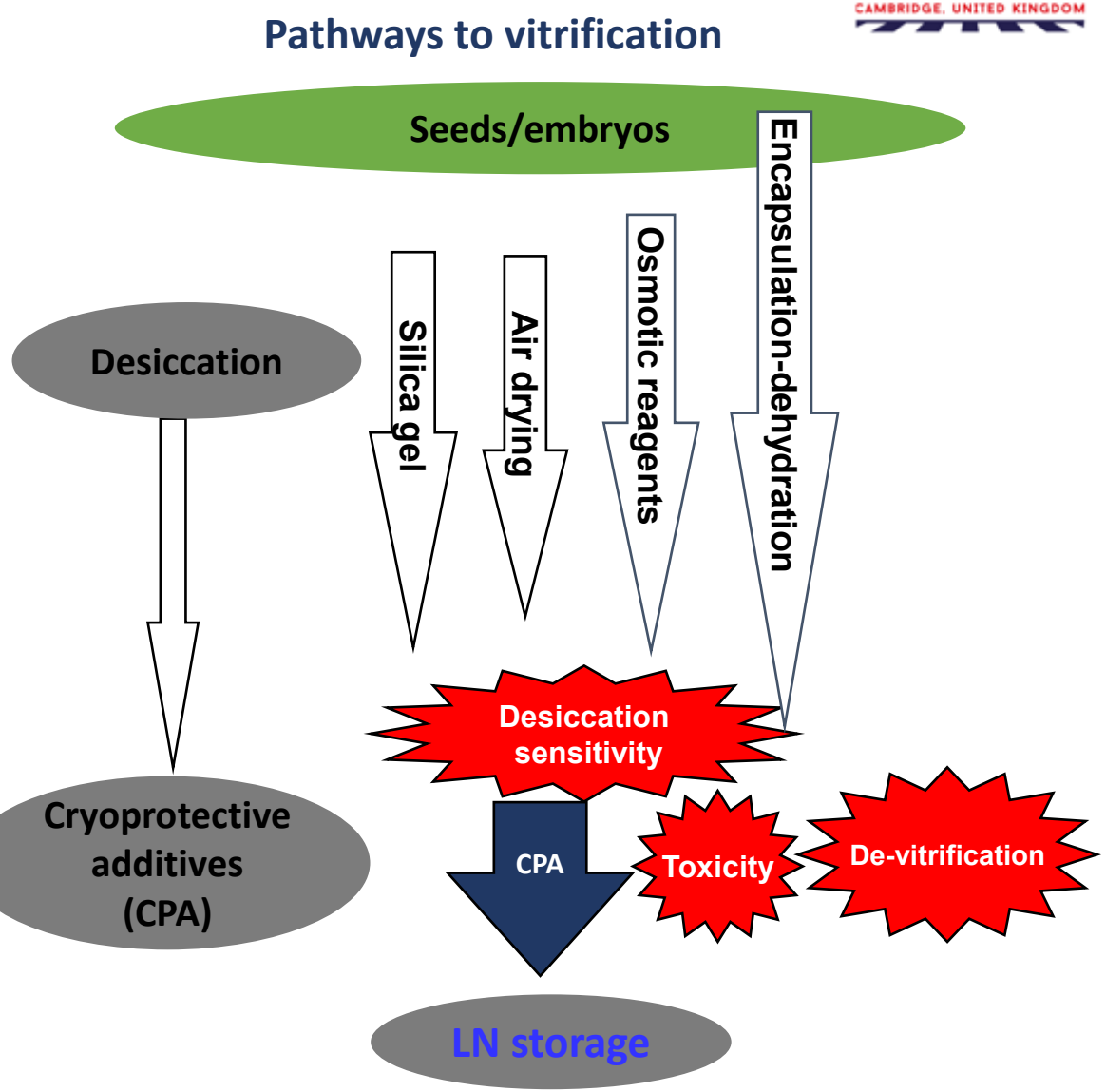
Dewars

Focus group

- Recalcitrant seed producing species
- Clonally propagated species – potato, cassava, banana, yam, sweet potato
- Intermediate and oily seed producing species – (Brassicaceae, Asteraceae, Cuphea, papaya, neem, citrus, orchids etc.)
- Orthodox seed species that are identified as ‘short-lived’ under seed bank storage condition

Different cryopreservation techniques

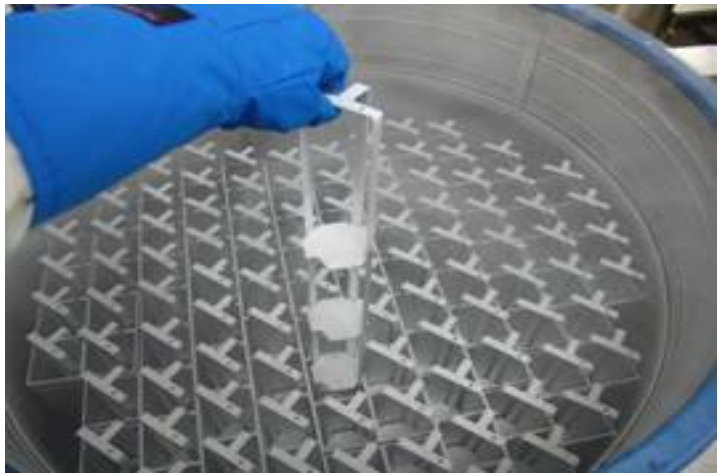
- Air drying followed by rapid freezing
- Encapsulation-dehydration
- Step-wise freezing
- Vitrification — using cryoprotective additives (CPA)
- Encapsulation-vitrification
- Droplet-vitrification
- Vacuum infiltration vitrification
- Cryo-plates
- Cryo-mesh
- Freezing using nitrogen slush at -207^{o}C



Critical factors in seed cryopreservation

- **Seed maturity**

- **Seed moisture content**
  - Most critical factor
  - Optimum moisture content must be attained
  - High or low create problems



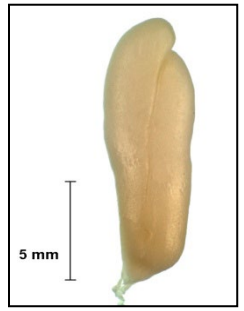
- **Cooling / rewarming rates**
  - A significant factor in survival

- **Physical damage to seed:**
  - If the seed matrices are capable of expanding and contracting with such cooling/rewarming rates
  - Depends on the size and surface area of the seeds.

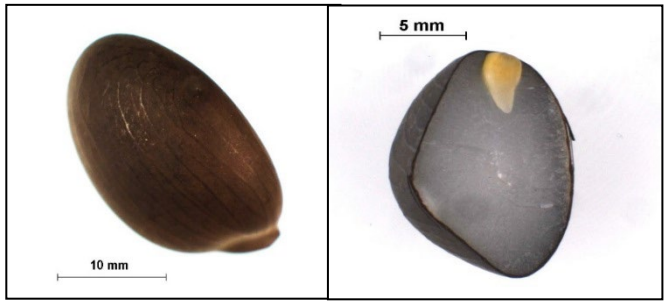
Recalcitrant seed cryopreservation – embryo & axis

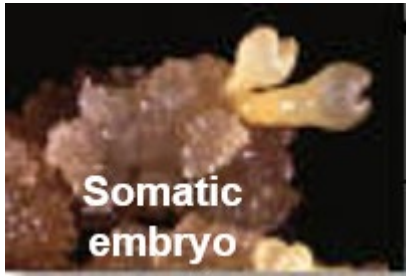
- Whole seed cryopreservation can only be applied to seeds of orthodox and intermediate (oily) seeds
- For desiccation sensitive seeds, excised zygotic embryos have been used
- Challenges are particularly significance for cryopreservation of recalcitrant seed species that produce large seeds
- These have to be handled as excised embryonic axes which poses additional challenge
- **Post-cryo recovery:** generally, involve *in vitro* manipulations (tissue culture, embryo rescue, somatic embryogenesis, shoot micropropagation) and need to be established prior to storage

Cycas revoluta

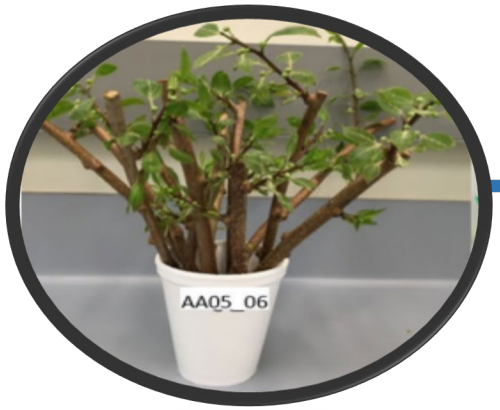


Trinax radiata



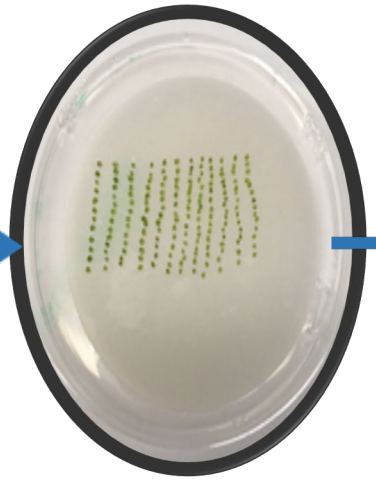


Shoot tip cryopreservation protocol



Shoot initiation into tissue culture from field sourced bud wood

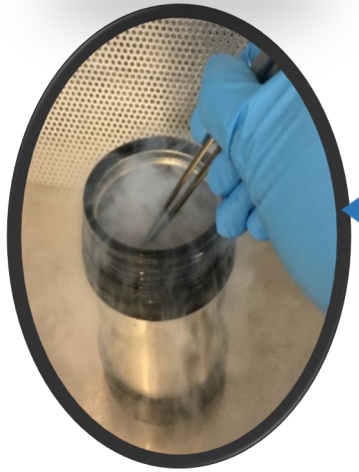
Plantlets multiplied in tissue culture



Explants- shoot tips (<1mm)

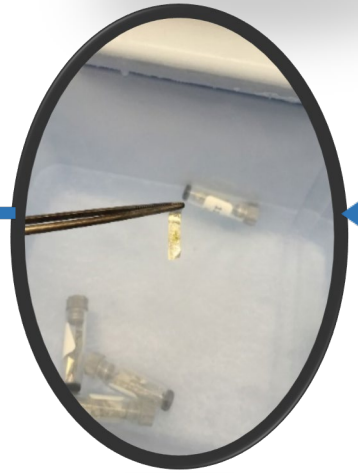
Cryoprotectants

Droplet in aluminium foil



Freezing in liquid nitrogen

Cryo-storage



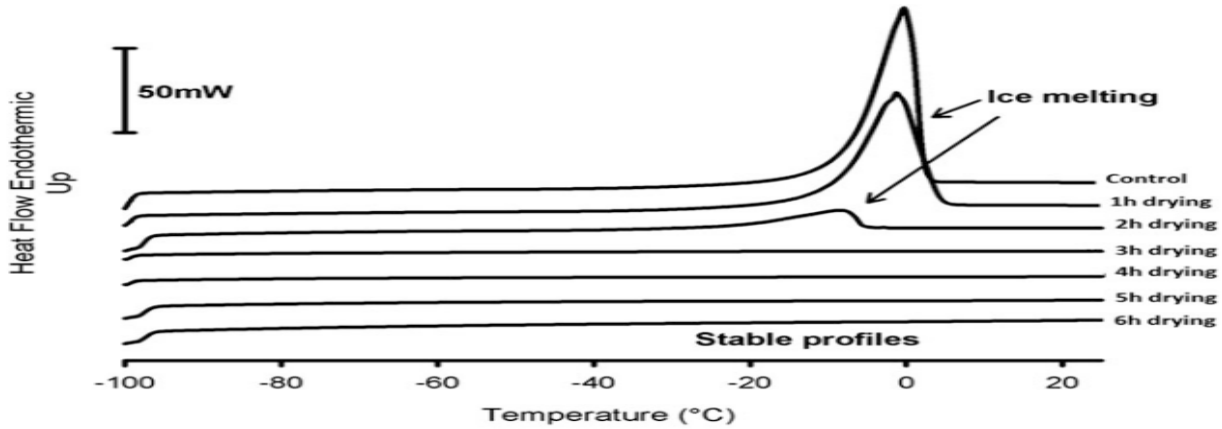
Thawing & washing off cryoprotectants

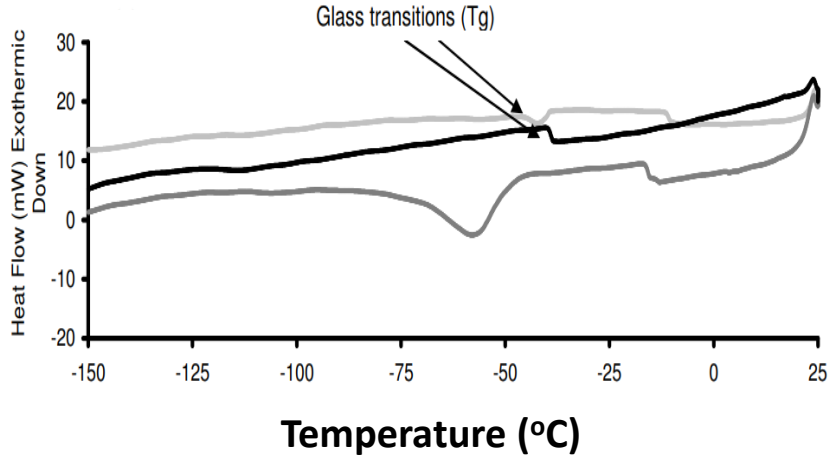
Regenerated plantlets after cryopreservation

Determination of glassy state using DSC thermal analysis

- Understanding the physical properties of water in seed tissues being stored at sub-zero temperatures is pivotal.
- Water-solute molecular properties provide information about ice formation potential, glass transition and vitrified state of the samples.
- DSC thermal analysis provides the feasibility to quantify freezable, non-freezable water and if the cells have reached vitrification.

Differential Scanning Calorimetry



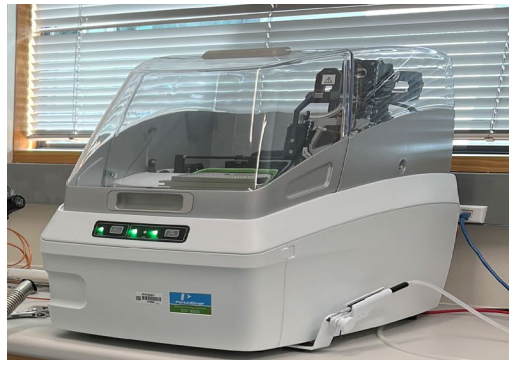


Nadarajan et al. CryoLetters 29(2),95-110 (2008)

Advancements in cryobiotechnology and cryo-engineering

- Biophysical studies to ensure glass stability can be achieved and sustained in cryo-banked materials
- Thermal analysis using DSC is applied to help to optimise vitrification-based cryoprotection protocols
- Modulated DSCs and Dynamic Mechanical Aanalysers have been used to identify glass transitions more accurately
- Programmable freezers are used for stepwise freezing
- Cryo-microscopy to visualise localised ice crystallisation
- Personalised cryoprotectant treatments
- Rapid freezing in slush nitrogen temperature at −207°C instead of in liquid nitrogen temperature at −196°C

Programmable freezer



Differential scanning calorimetry

Dynamic mechanical analyser (DMA)

Advantages of cryopreservation

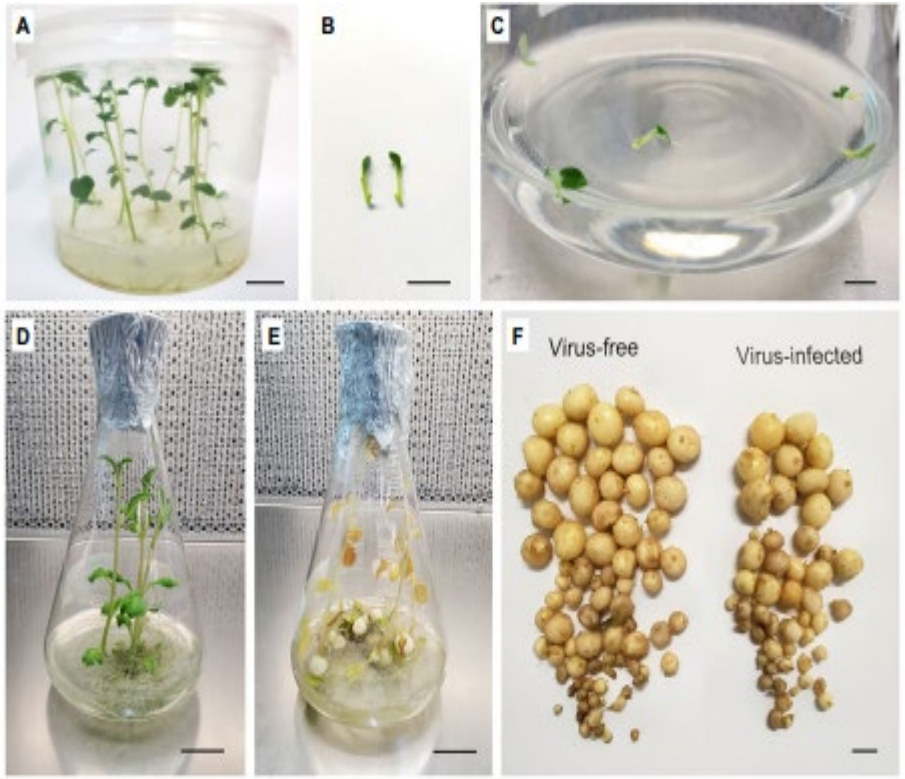
- Provides long-term storage for plant germplasm
  - Projection for cryo-longevity: Half-life of lettuce seed = **c. 500 years (vapour phase above LN)** and c. **3400 years (in LN)** c. 50 – 75 times longer lived than seeds stored at -20°C.Walters et al., 2004 (*Planta* 48, 229-44)
- Genetically stable material
- A complementary storage method for difficult to store materials
- Reduces metabolic seed deterioration. All metabolism would be essentially stopped and extend storage life
- Liquid nitrogen in self-contained tanks, not dependent on mechanized refrigeration
- Less labour and place requirements, more economical
- Virus eradication/fungal-free plants materials

Virus eradication using cryotheraphy

Eradication of *Potato Virus S*, *Potato Virus A*, and *Potato Virus M* From Infected *in vitro*-Grown Potato Shoots Using *in vitro* Therapies

Jean Carlos Bettoni[1]‡, Liya Mathew[1‡], Ranjith Pathirana[1†], Claudia Wiedow[1], Donald A. Hunter[1], Andrew McLachlan[1], Subuhi Khan[2], Joe Tang[2] and Jayanthi Nadarajan[1]*

Front. Plant Sci., 19 May 2022
Sec. Plant Biotechnology
Volume 13 - 2022 | https://doi.org/10.3389/fpls.2022.878733



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Thank You

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