DanSeed Symposium

Current Developments in Seed Cryopreservation

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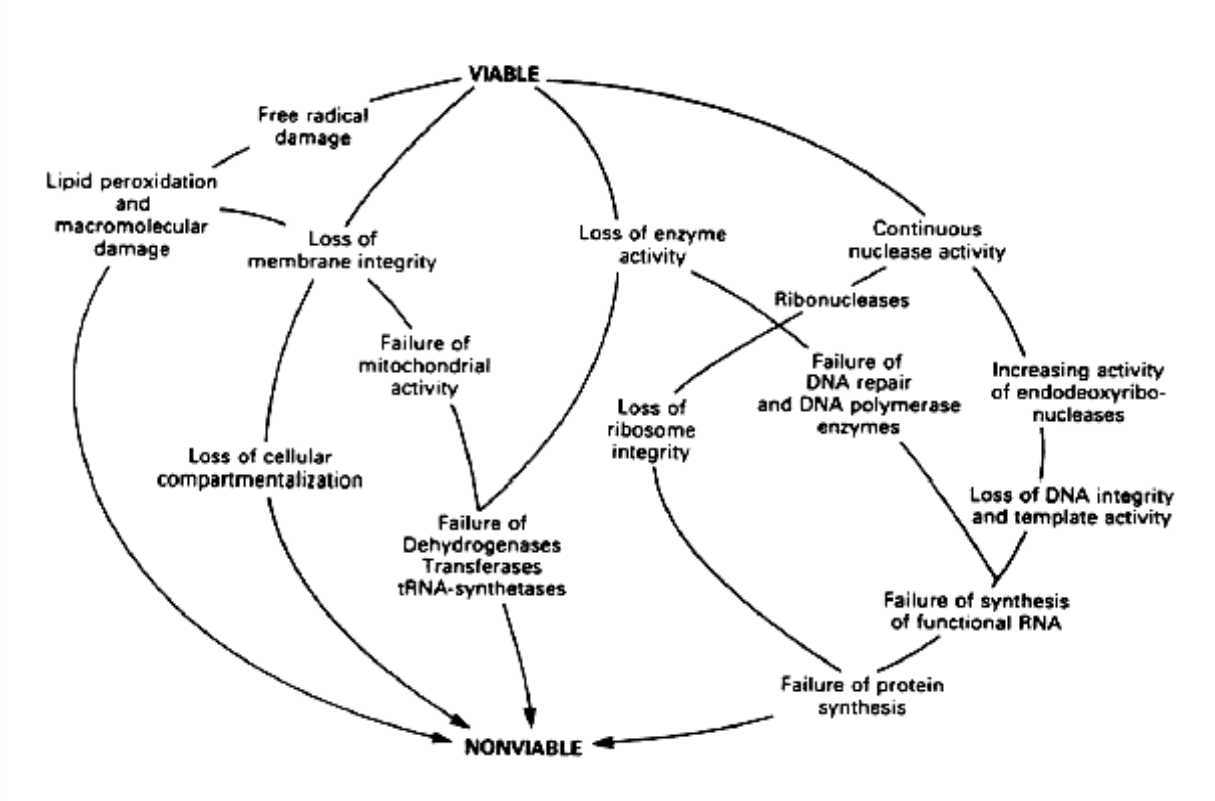
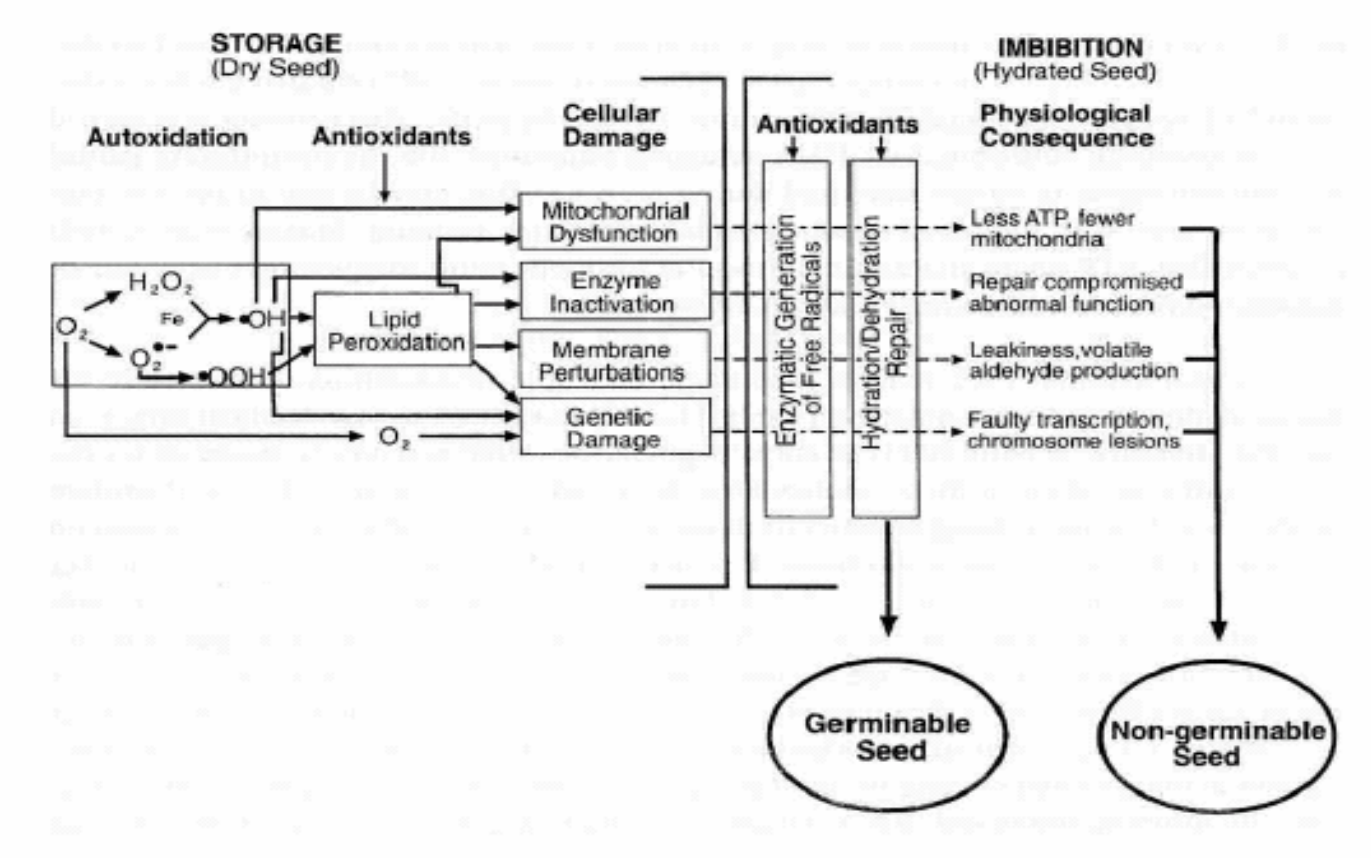
Loss of seed viability



From Osborne 1980

Decline in seed germination



From McDonald 1999

Aim of seed storage

to minimise deterioration it is essential to minimise water activity by maintaining free water remaining in the seed in a vitrified state during storage...

.and avoid subsequent damage when devitrification occurs on withdrawal from storage...

- Challenges
  - ❖ Achieving vitrification to minimise water activity
  - ❖ Problematic lipid phase changes

Vitrification

Vitrification - water makes the phase transition from liquid to the amorphous glass state, characterised by high viscosity and the absence of crystalline structure. Occurs with high desiccation in an orthodox seed

Vitrified water sustains minimal aqueous chemistry, and even less so with reduced temperature.

Reduction in water activity

Table 1. Water activity, water potential (eq. 1), and water contents of rape (*Brassica napus*) and wheat (*Triticum vulgare*).

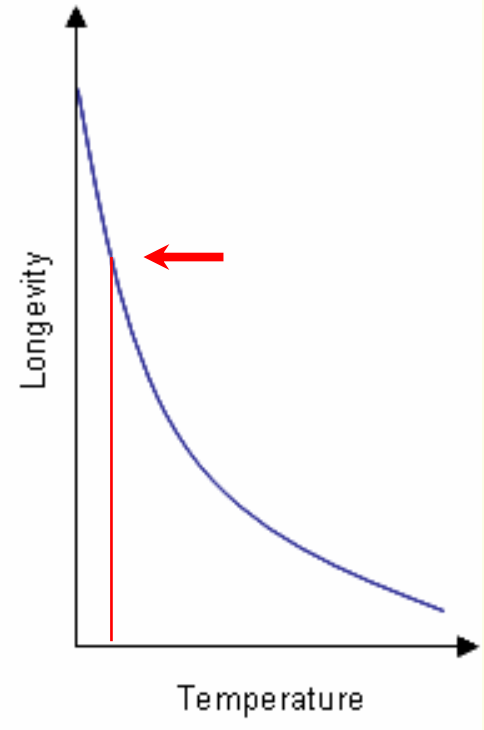
Water Activity	Water Potential (MPa)	Rape Water Content (g/g)	Wheat Water Content (g/g)
0.10	-314	0.031	0.060
0.20	-219	0.039	0.080
0.30	-164	0.045	0.093
0.40	-125	0.052	0.106
0.50	-94.4	0.060	0.120
0.60	-69.6	0.069	0.132
0.70	-48.6	0.080	0.147
0.80	-30.4	0.093	0.163
0.90	-14.3	0.121	0.215

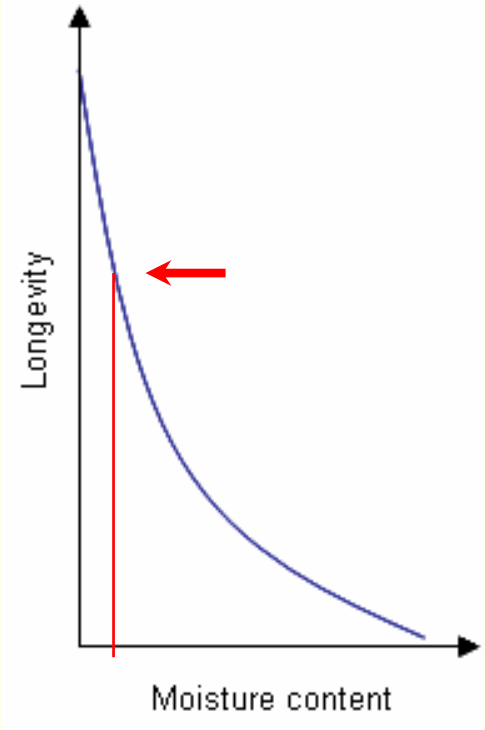
Water content data are from Roberts (1972).

Orthodox seed: conventional storage

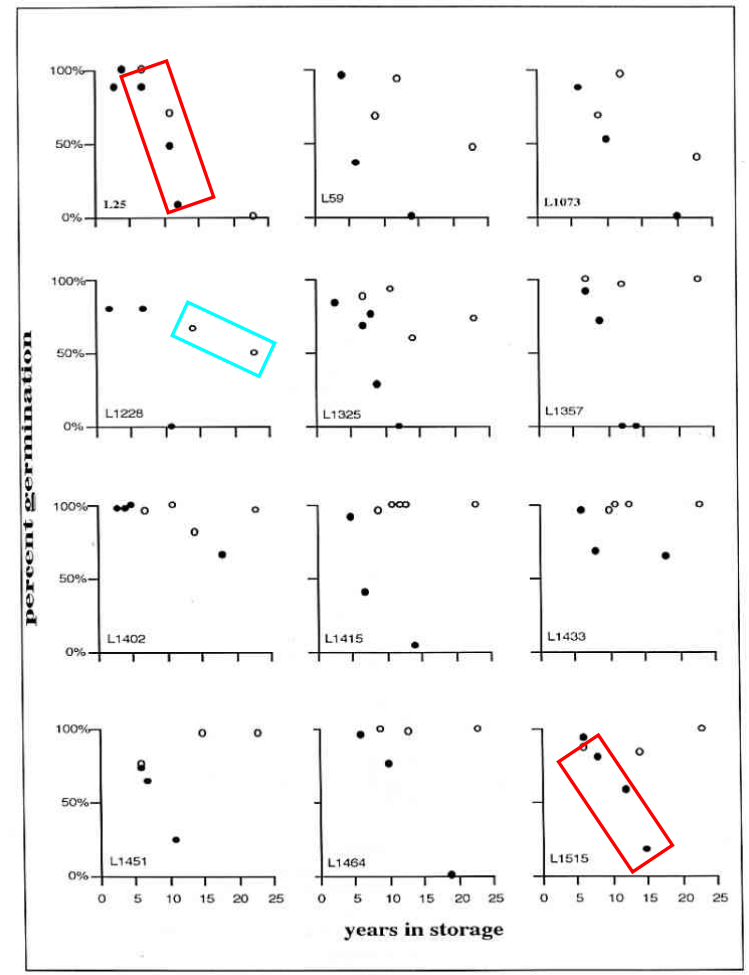
Vitrification by drying – relatively stable below c10^{0}C in orthodox seeds

log σ = *KE - CW* log *m - CH T - CQ T2 (Ellis & Roberts 1980)*





Orthodox seeds



- Easily dried to low water content ~ 5%FW
- Moderate storage at cool room temperatures 5^0C
- Suitable for conventional freezer storage -18^0C
- Variation between seed lots
- Arguments for cryostorage – minimal problems

Recalcitrant & intermediate seed

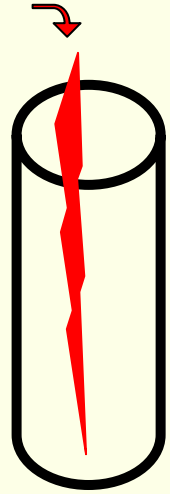
Vitrification by simple desiccation is not possible

Recalcitrant dessication intolerant ~ 20-45% FW
[Genetic]

Intermediate problematic in frozen storage – includes seeds which are readily dried and then lose viability more rapidly in storage. High oil content
[Developmental/environmental/genetic)

Excised embryos from difficult subjects
(embryo rescue/biotech. interest eg secondary embryos]

Vitrification at ultralow temperatures



[1]

<u>very rapid cooling rate</u>

direct immersion

PVS2 with toxicity problems

physical problems with some seed

Vials – cooling rate c200^{0}Cmin^{-1}

[2]

<u>encapsulation with dehydration</u>

serial dehydration of excised embryos/immature seed with osmotica

air desiccation ?

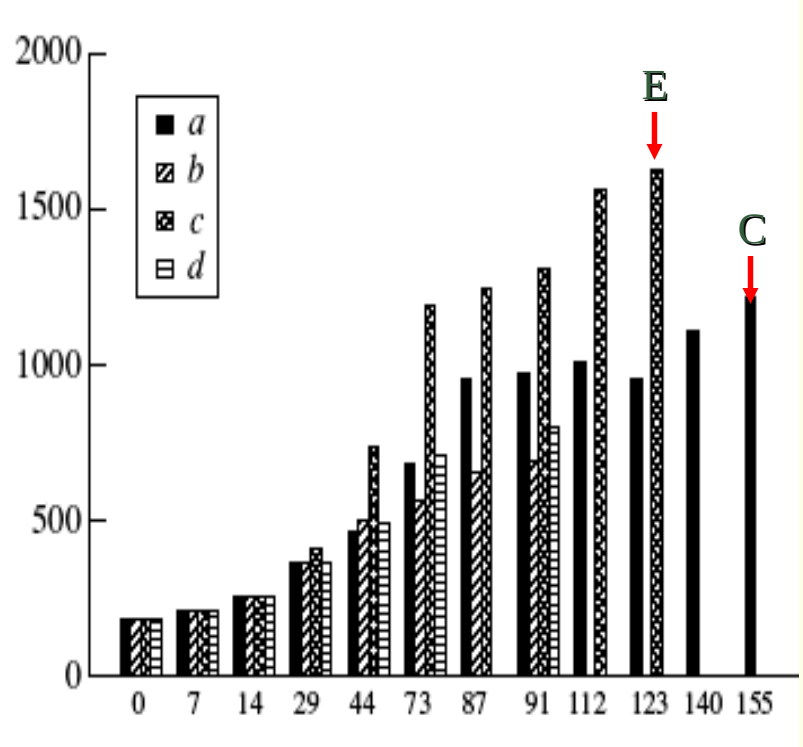
immersion into LN in vials c 200^{0}C min^{-1}

Some high oil seeds need slower cooling (Agathus, Pinus), perhaps related to the destabilisation of lipid glass phases

Immature Orchid Seed

Vitrification of small seed

Mature seed of *Calanthe vestita*
Fully dried
(Nikishin et al., 2000)

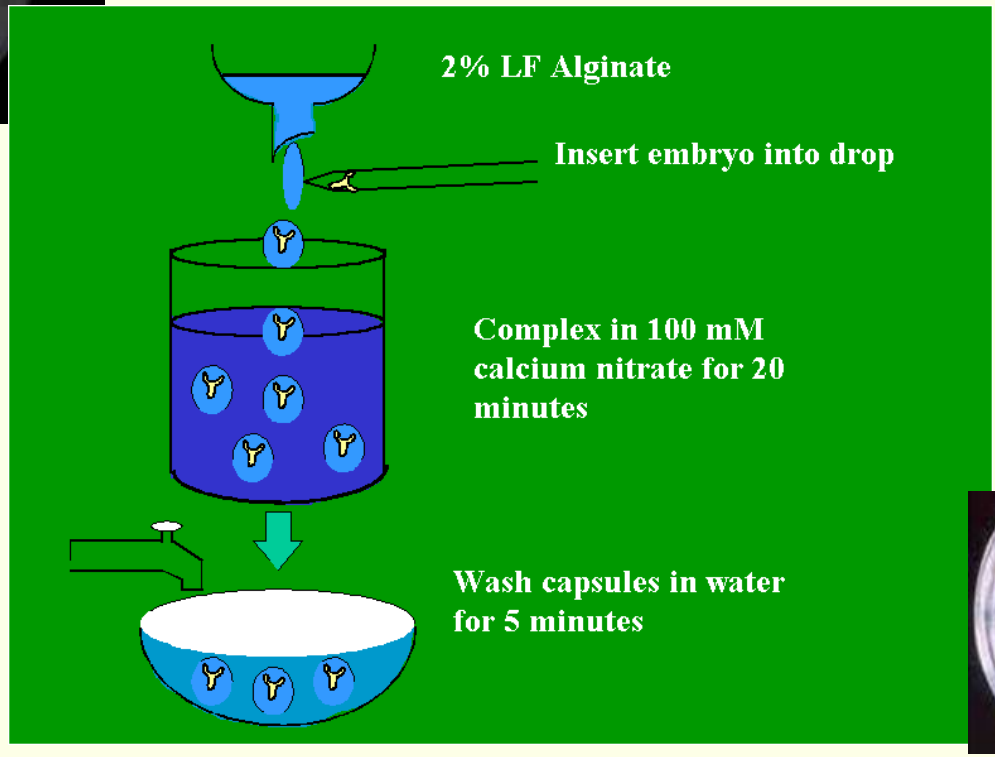


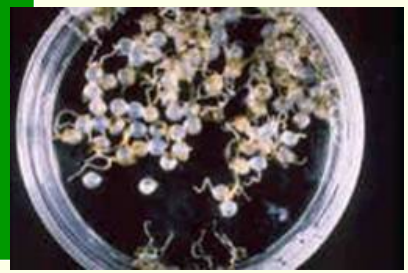
Immature seed of *Ponerorchis graminifolia*
(Hirano et al., 2005)

PVS2
30% w/v glycerol, 15% w/v ethylene glycol
14% w/v sucrose,15% v/v DMSO
Growth medium

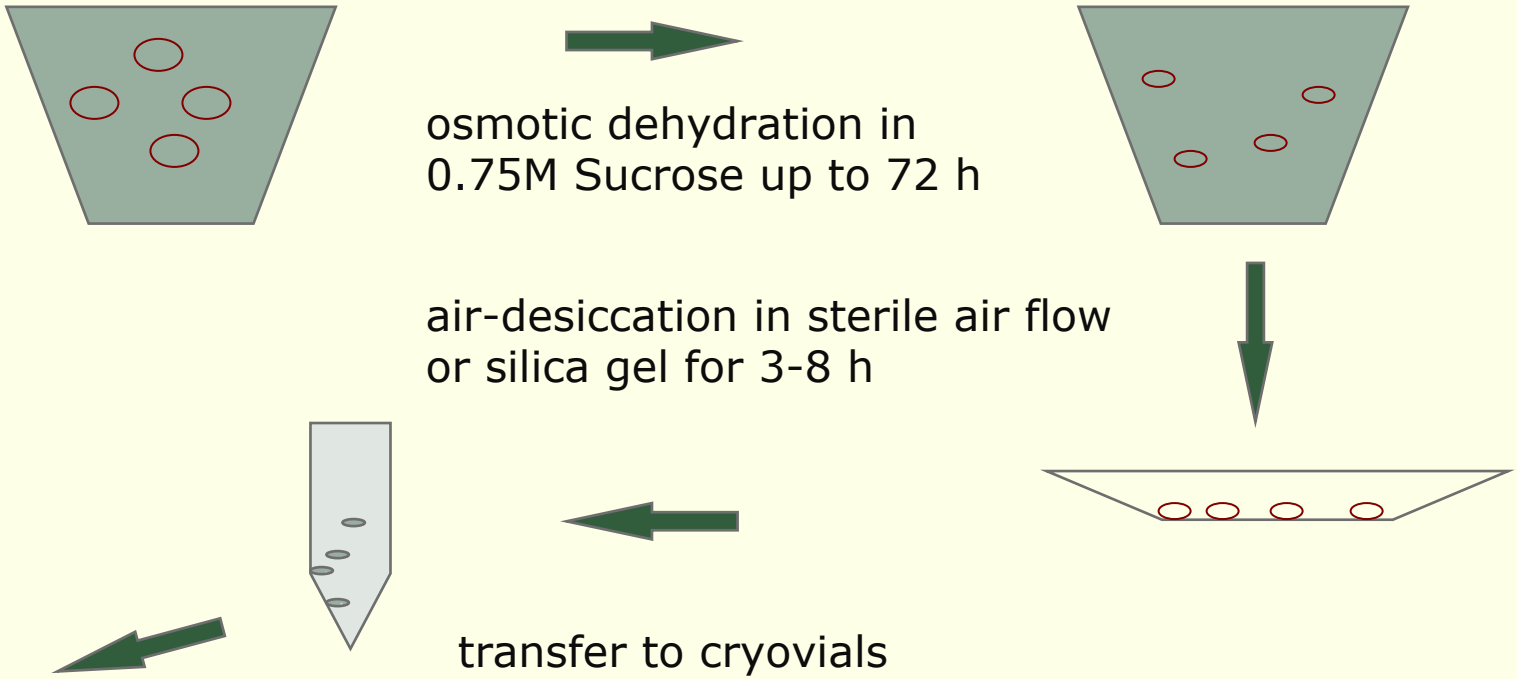
	6 / 9 WAP	**6 / 9 WAP**
	% germination	% protocorm
control	51.5[a] / 19.2[a]	12.9[a]/ 1.8[b]
LN vitrify	50.1[a] / 19.0[a]	19.0[a] / 0.0

Alginate encapsulation





Encapsulated vitrification



Encapsulated orchid

Dactylorhiza fuchsii seed (common spotted orchid) encapsulated in alginate beads with the fungal symbiont *Ceratobasidium cornigerum*.

Beads dried to 20% FW moisture content

(Wood et al., 2000)

Seed and symbiont germination/growth

°C	3 days	30 days
16	62 73	15 32
-20	12 0	10 0
-70	54 56	54 52
-196	96 100	96 100

Cryostorage losses

Table 3
Seed germination following storage in liquid nitrogen (−196 °C) for 10–20 years

Species	# Accessions tested	Percentage germination				Storage time (years)
		Initial (95% CI)		Final (95% CI)		
Significant trend ($P < 0.05$)						
Abies procera	4	56.3	41.6	53.1	16.5	11.7
*Allium cepa**	21	95.6	90.3	90.6	79.6	10.4
*Beta vulgaris**	11	94.5	88.9	79.2	53.8	13.7
Eragrostis curvula	3	96.7	93.9	82.6	53.6	14.2
Helianthus annuus	3	95.1	91.5	92.9	74.7	20.4
*Lactuca sativa**	9	98.6	97.7	97.3	93.8	12.1
Medicago sativa	4	93.0	84.6	87.1	77.5	13.4
Papaver somniferum	3	99.4	88.9	94.6	51.6	14.2
Petroselium crispum	2	85.0	76.9	74.8	60.0	21.2
Ulmus americana	1	89[a]		77[a]		21.6
*Vicia spp.**	3	95.6	92.4	88.6	84.8	14.2
*Zinnia violacea**	4	91.2	88.3	87.8	78.3	13.7
No significant trend ($P > 0.05$)						

Lettuce seed: -18^{0}C 46 - 70 yrs
-196^{0}C 500 yrs vapour; 3400 yrs liquid

Lipid problems

For seeds at higher moisture content, and particularly with a high lipid content also, there is a risk that, small quantities of injurious ice could form during cooling / warming.

This may be due to lipid phase changes that enable v small, benign ice crystals to coalesce into damaging ones

Lipid coalescence on warming

Warming and rehydration

Particularly important for oilseeds

In *Coffea* there is a significant correlation between unsaturated fatty acid levels and successful germination and growth after cryopreservation

Recovery can be aided by high temperature treatments 35°C prior to gerrmination eg *Coffea* and *Azadirachta.*

The mechanism of action is presumed to be by facilitating gel-to-liquid crystalline phase transitions in membranes.

Warming rate and water content

Warming rate to achieve optimal survival has a relationship with the water content of the original material

Appropriate warming can extend the moisture content range for survival

Rye grass warmed from LN:

at 30°C min-1 survival up to 19% FW as pre-freeze water content

at 1500 °C min-1 level raised to 23% FW(Sakai and Noshiro 1975)

16% to 21%FW for rice under similar conditions

In summary

Least problems with orthodox seeds with low oil/lipid levels, and storage life can be predicted reliably

Intermediate and recalcitrant seeds may need vitrification with or without encapsulation. *In vitro* technology is required and storage is less predictable

Lipid changes during cooling, storage and warming make the situation more complex than simply managing water

Longer germination time to allow for repair of non-lethal injury

Thank you for listening…..

your questions & comments are welcome

Tak for jeres opmaerksomhed
spørgsmål og kommentarer er velkomne

Acknowledgements: Hugh Pritchard and Jayanathi Nadarajan at the Millennium Genebank, Wakehurst Place, Kew Gardens, UK ; Erica Benson, University of Derby, UK