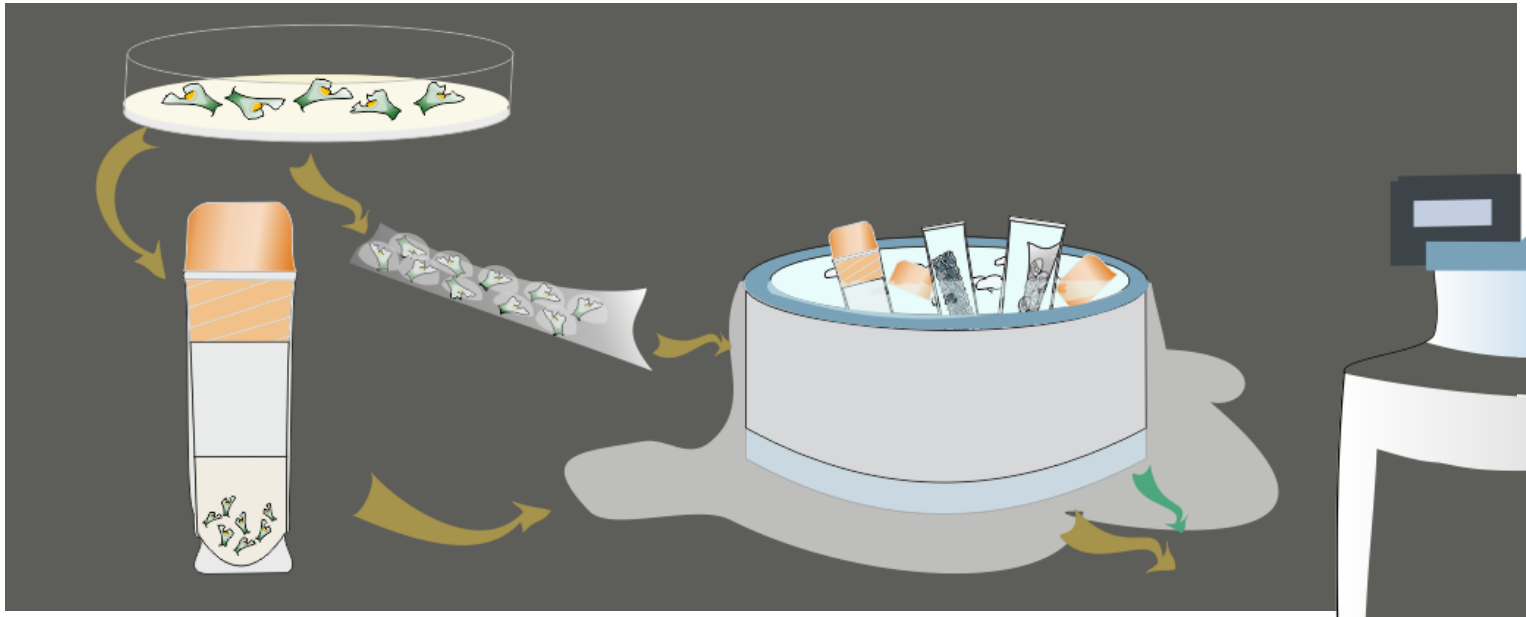
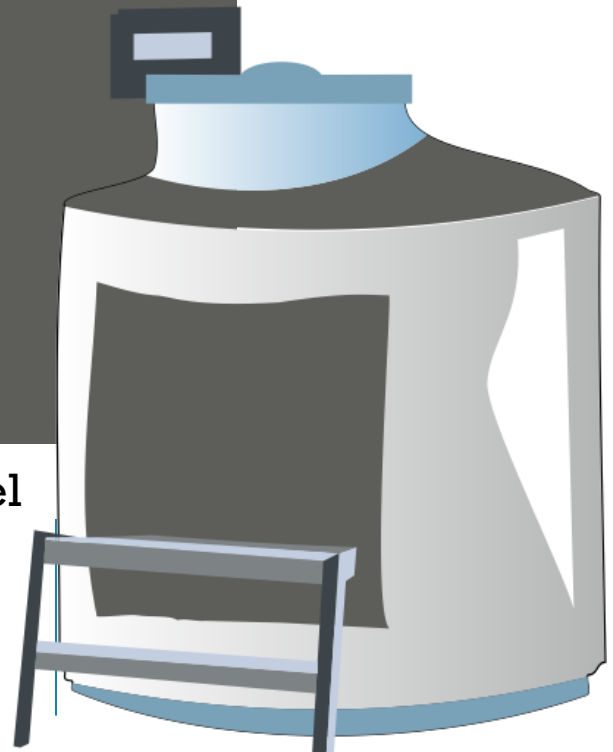
Plant Cryopreservation
Principles, Applications and Challenges of Banking Plant Diversity at Ultralow Temperatures



PD Dr. Manuela Nagel



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PGR maintenance at IPK

Seed cold storage (~145,000 accessions)

Field genebank (1,380 accessions)

Slow-growth storage (3,130 accessions)

Cryo (2,500 accessions)

IPK, 2025

Weise et al. (2025) Genetic Resources S2; https://doi.org/10.46265/genresj.GYDY5145

Photos: S. Rey (2016), L. Maine (2016), M. Nagel (2019)

Thanks to the co-authors

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ANNUAL REVIEWS

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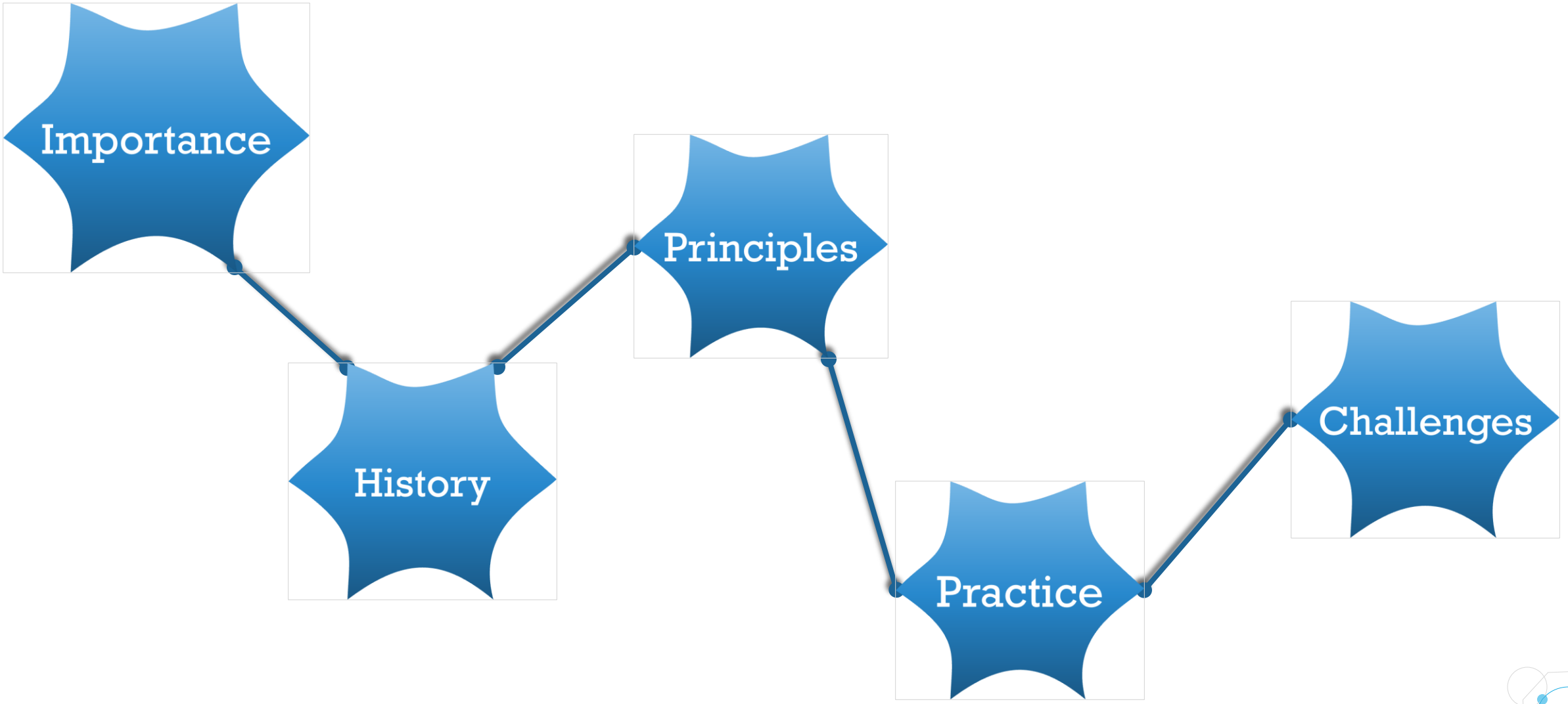
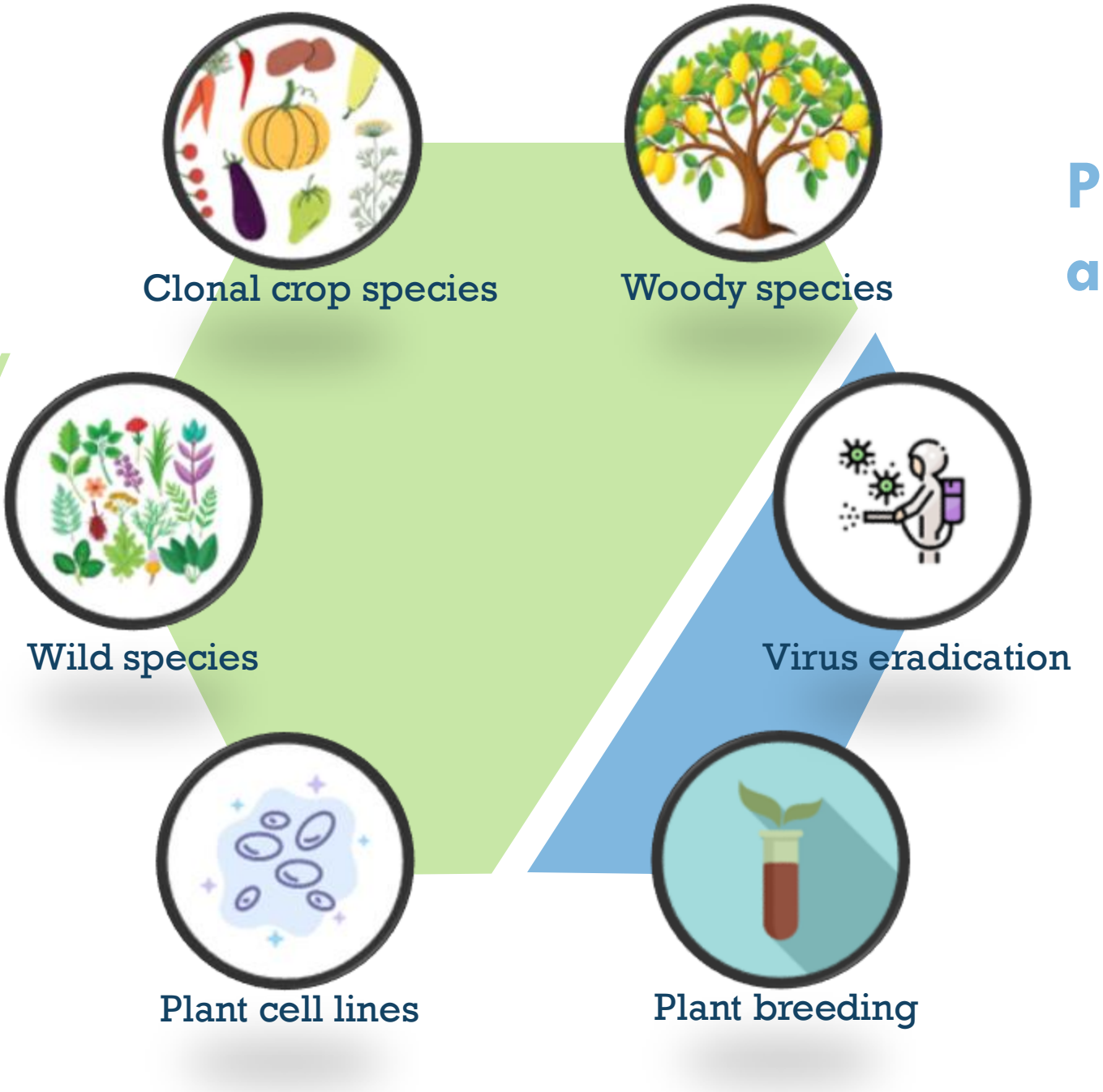
Structure



Why is plant cryopreservation important?

Importance for

Conservation of genetic diversity



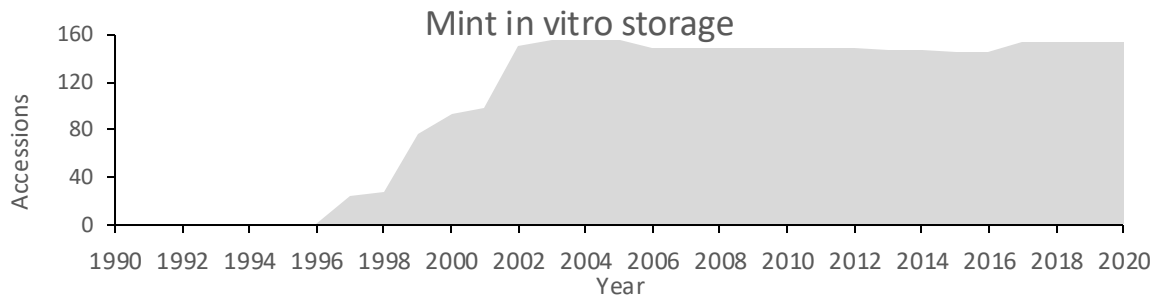
Plant breeding and production

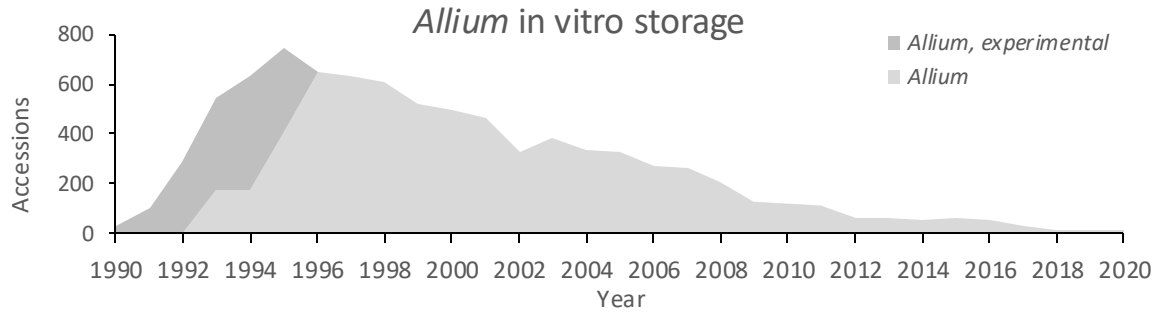
Field maintenance

- Accumulation of mutations & endophytic organisms
- Exposure to adverse weather conditions
- Exposure to pathogens and insects
- Plant ageing

Panis et al. (2020) Plants 9; https://doi.org/10.3390/plants9121634
Photo: Manuela Nagel (2019)

In-vitro Slow-growth storage

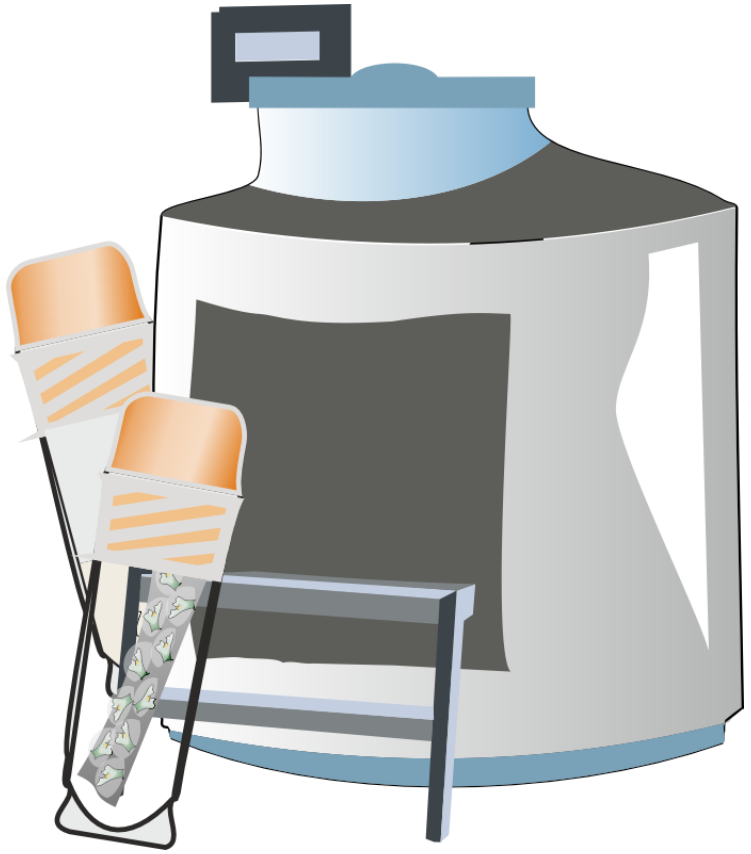




- In-vitro-recalcitrance
- Plant ageing
- Somaclonal variations
- Specific protocol development
- Infestations of insects & contaminations

Weise et al. (2025) Genetic Resources S2; https://doi.org/10.46265/genresj.GYDY5145
Panis et al. (2020) Plants 9; https://doi.org/10.3390/plants9121634
Photo: L. Main (2016)

Cryopreservation



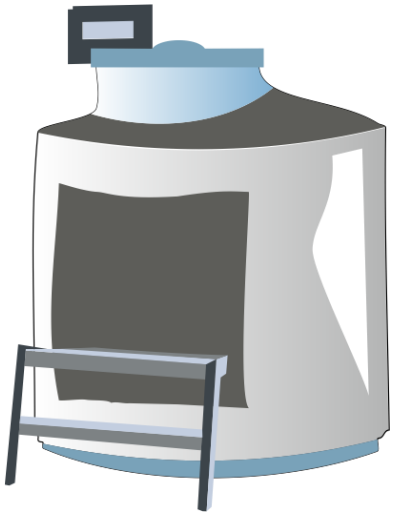
- High genetic stability
- Minimum space requirements
- Low long-term costs
- Long-term storage of >100 years

Panis et al. (2020) Plants 9; https://doi.org/10.3390/plants9121634

Need for cryopreservation

Field genebanks
~400,000 accessions

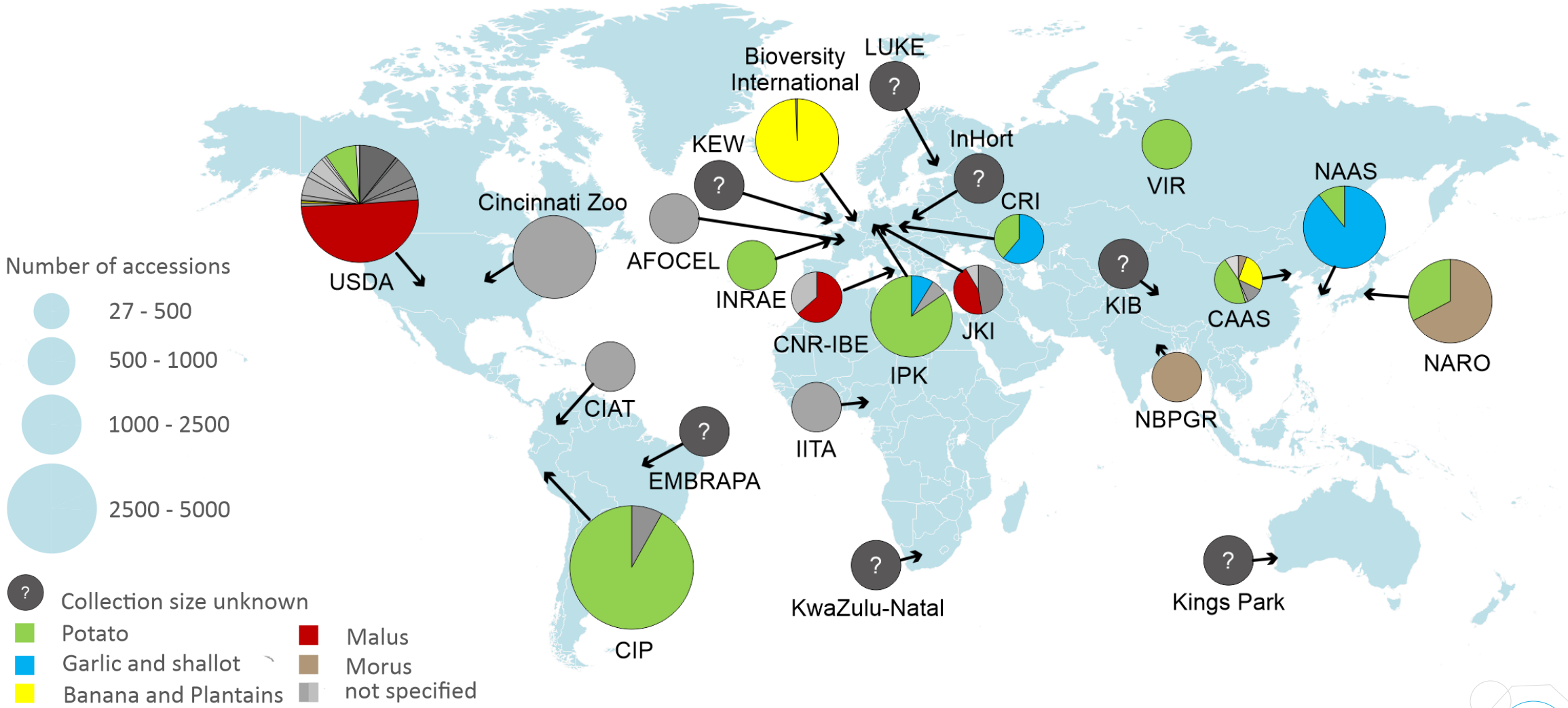
In vitro genebanks
~33,000 accessions



Cryobanks
~17,690 accessions

Nagel et al. (2024) Annual Reviews of Plant Biology 75, 797-824 https://doi.org/10.1146/annurev-arplant-070623-103551
Panis et al. (2020) Plants 9; https://doi.org/10.3390/plants9121634

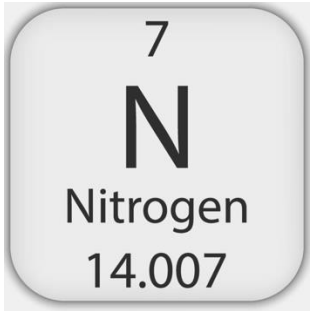
World Cryobanks

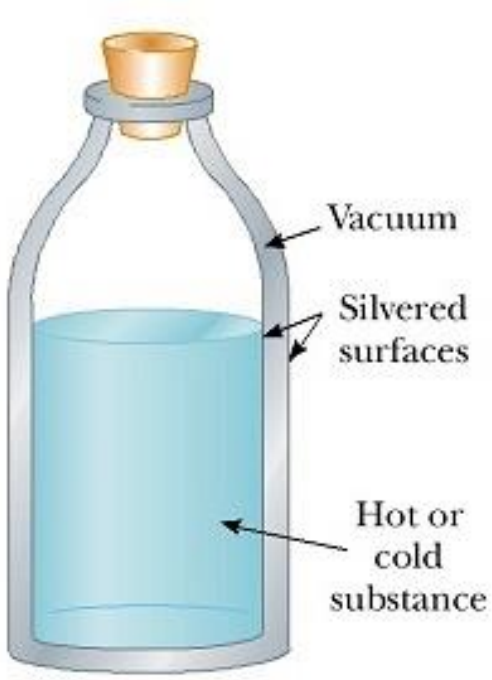


Nagel et al. (2024) Annual Reviews of Plant Biology 75; 797-824 https://doi.org/10.1146/annurev-arplant-070623-103551

What is the historic background for cryopreservation?

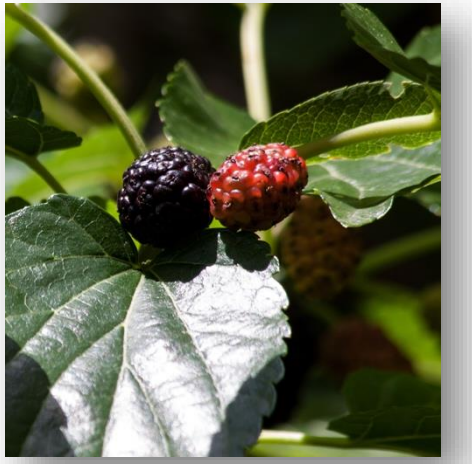
Liquid nitrogen - cryogenics





- Discovered by Daniel Rutherford in 1772
- inert gas, colourless, odourless, non-corrosive, non-flammable, very cold and cheap
- Atmosphere: 78% N_2, 21% O_2, 1% other components
- Boils at -196°C and freezes at -210°C
- In 1883, Zygmunt Wróblewski and Karol Olszewski discovered a method to liquidfy N_2 by compressing and cooling
- In 1898 James Dewar invented a tank

Akira Sakai (1960) experiments with mulberry buds



Pre-freezing for 16 hours 0

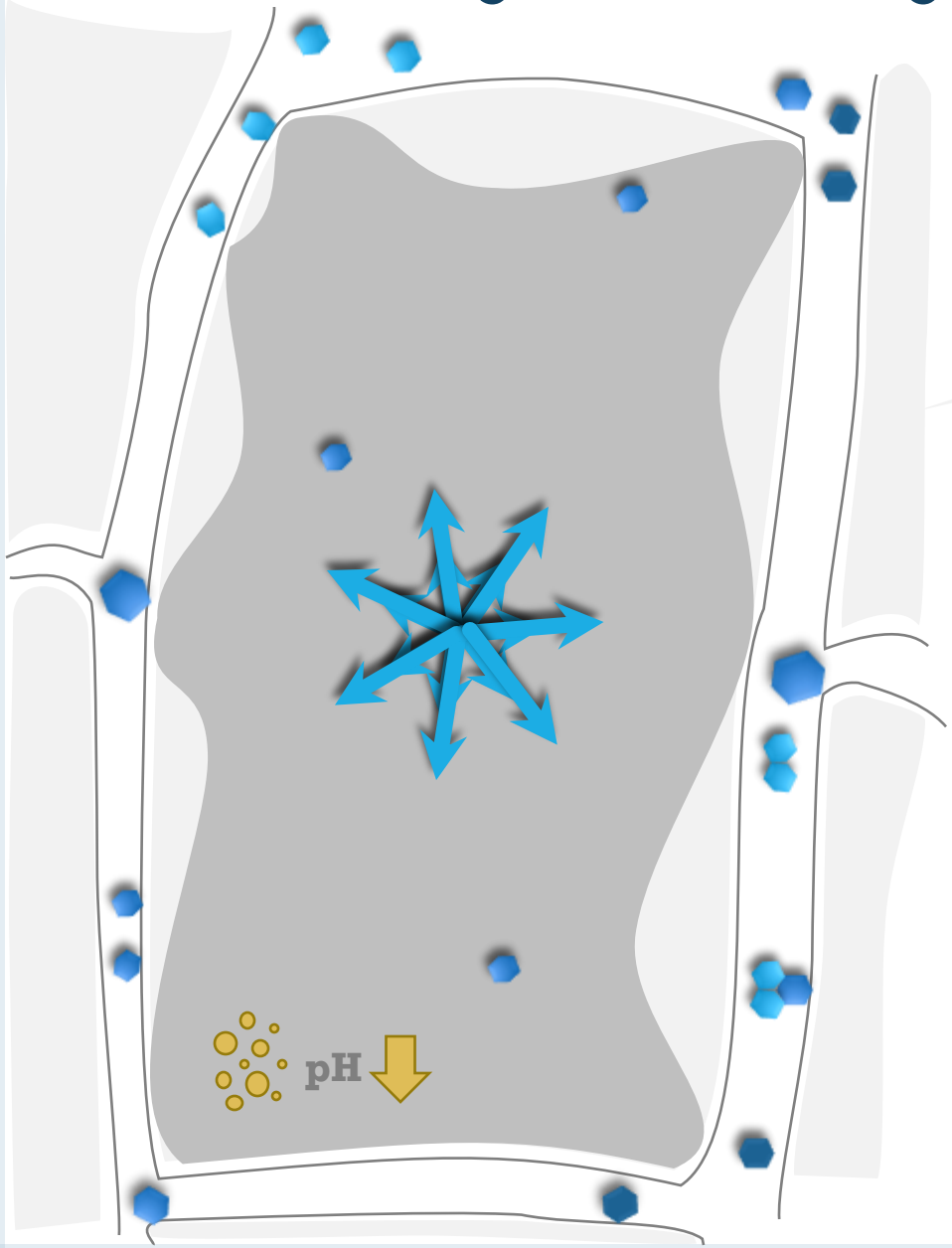
10 min LN

Rewarming for 4 hours

Survival 0

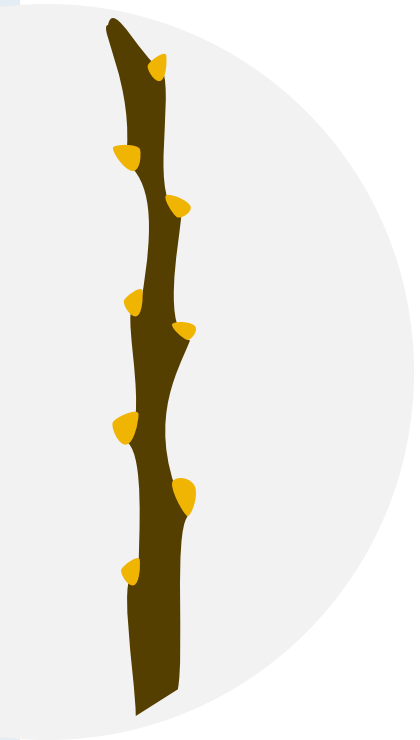
Sakai (1960) https://doi.org/10.1038/185393a0

Events during slow cooling of dormant winter buds

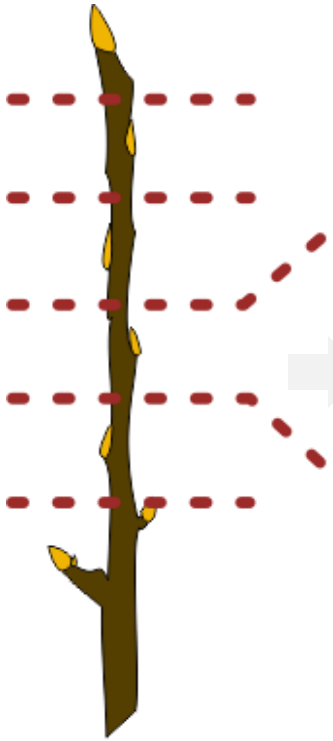


1. Cytoplasm supercools above -10°C
2. Extracellular water crystallize and water pressure falls
3. Electrolytes will precipitate, pH changes
4. All free water is converted to ice
5. Small crystals tend to form large crystals (recrystallisation)

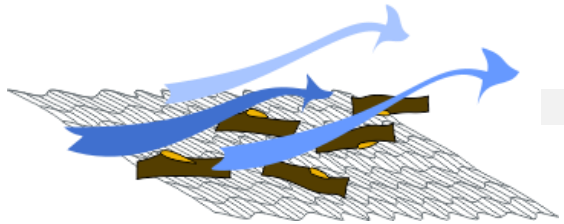
Development of dormant bud cryopreservation



Sampling in mid-winter



Uni-nodal segments, ~ 3.5 cm



Air dehydration at -5°C ~22% water content

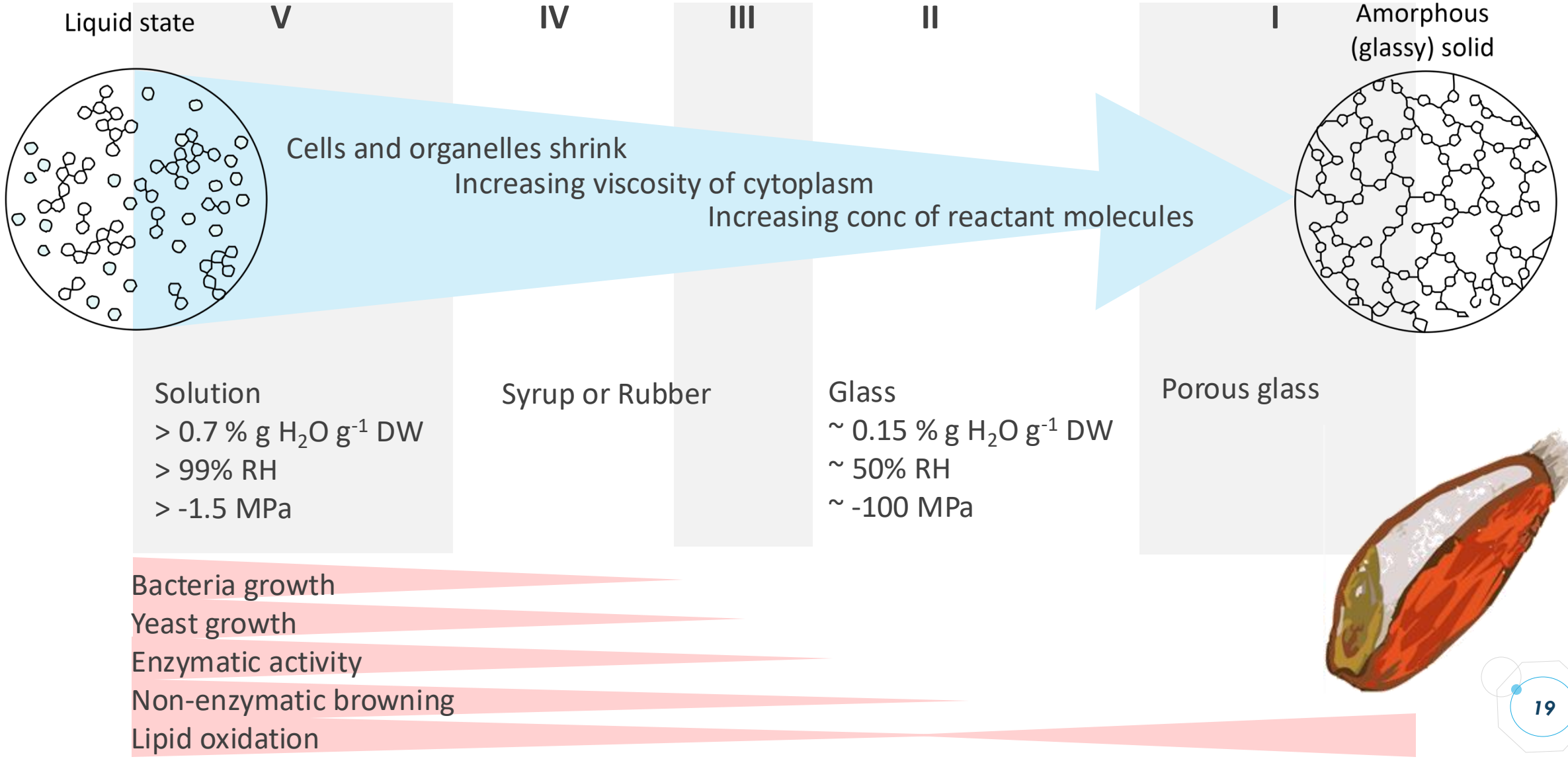
Cooling rate at 1°C h^{-1} to -30°C

LN storage

Nagel et al. (2024) Annual Reviews of Plant Biology 75; 797-824 https://doi.org/10.1146/annurev-arplant-070623-103551

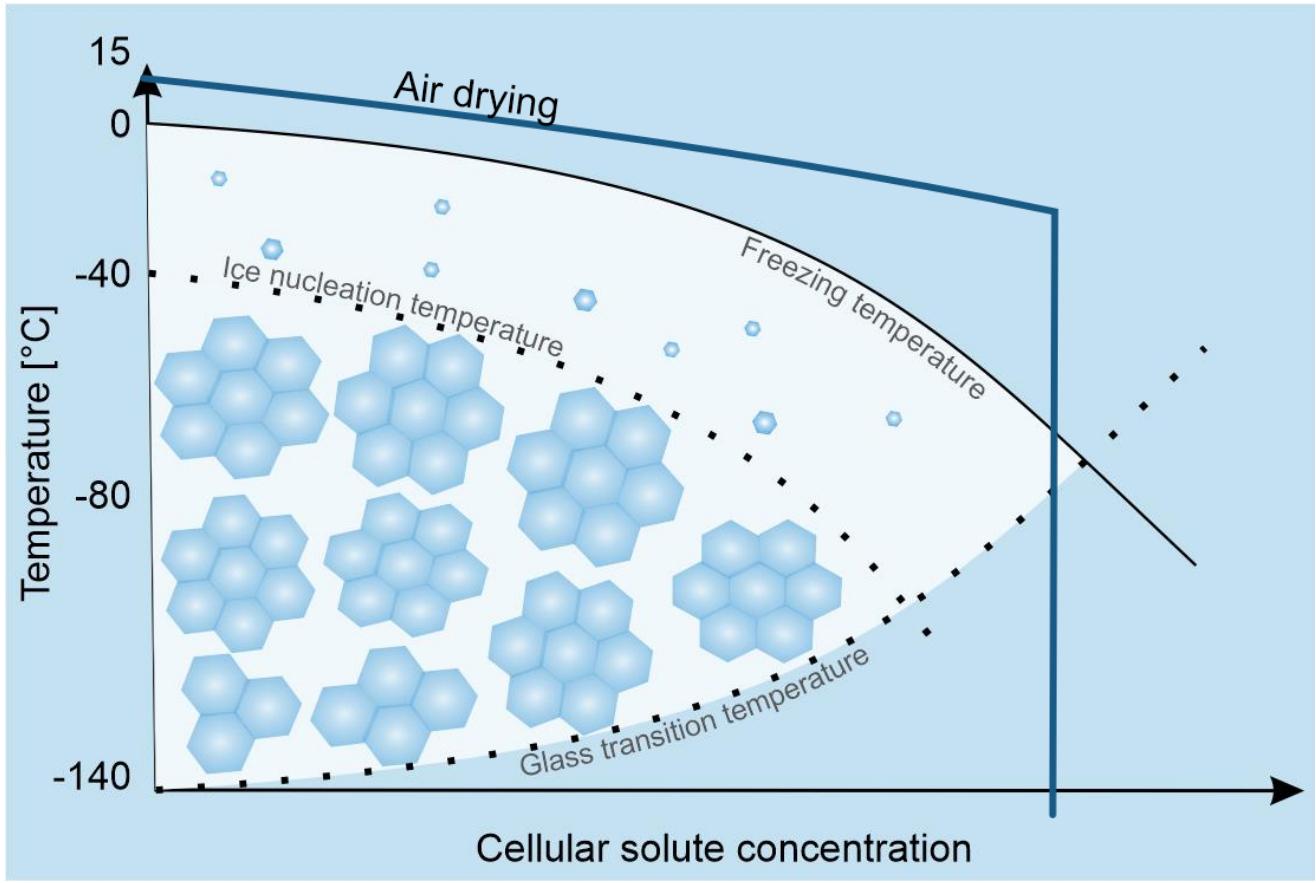
How to conserve organs in a viable state?

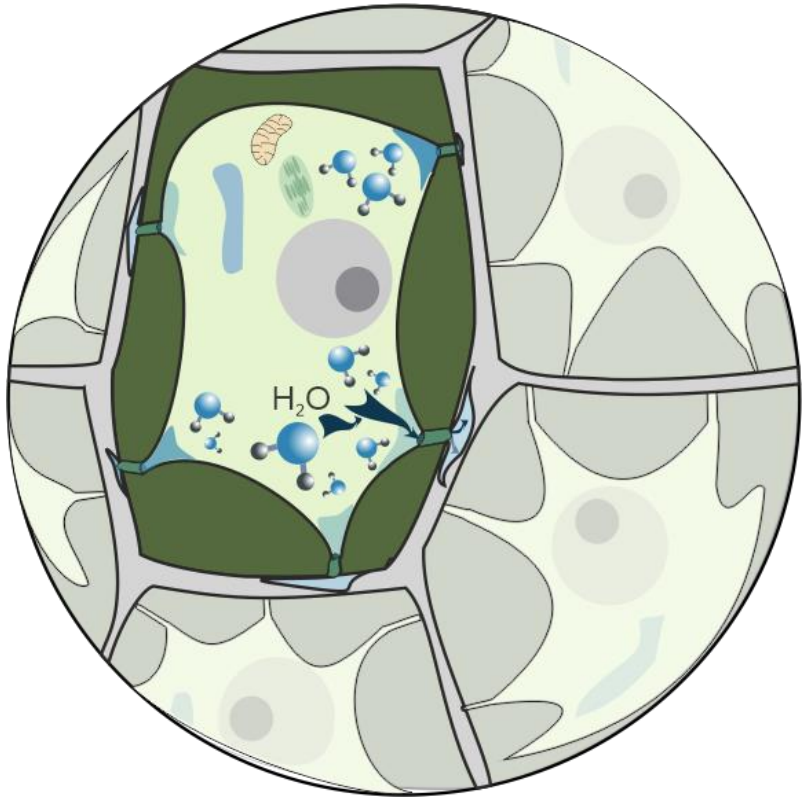
Storage at the glassy state



Walters et al. (2005). Integrative and Comparative Biology 45, 751-758.

Air drying





Ice crystals

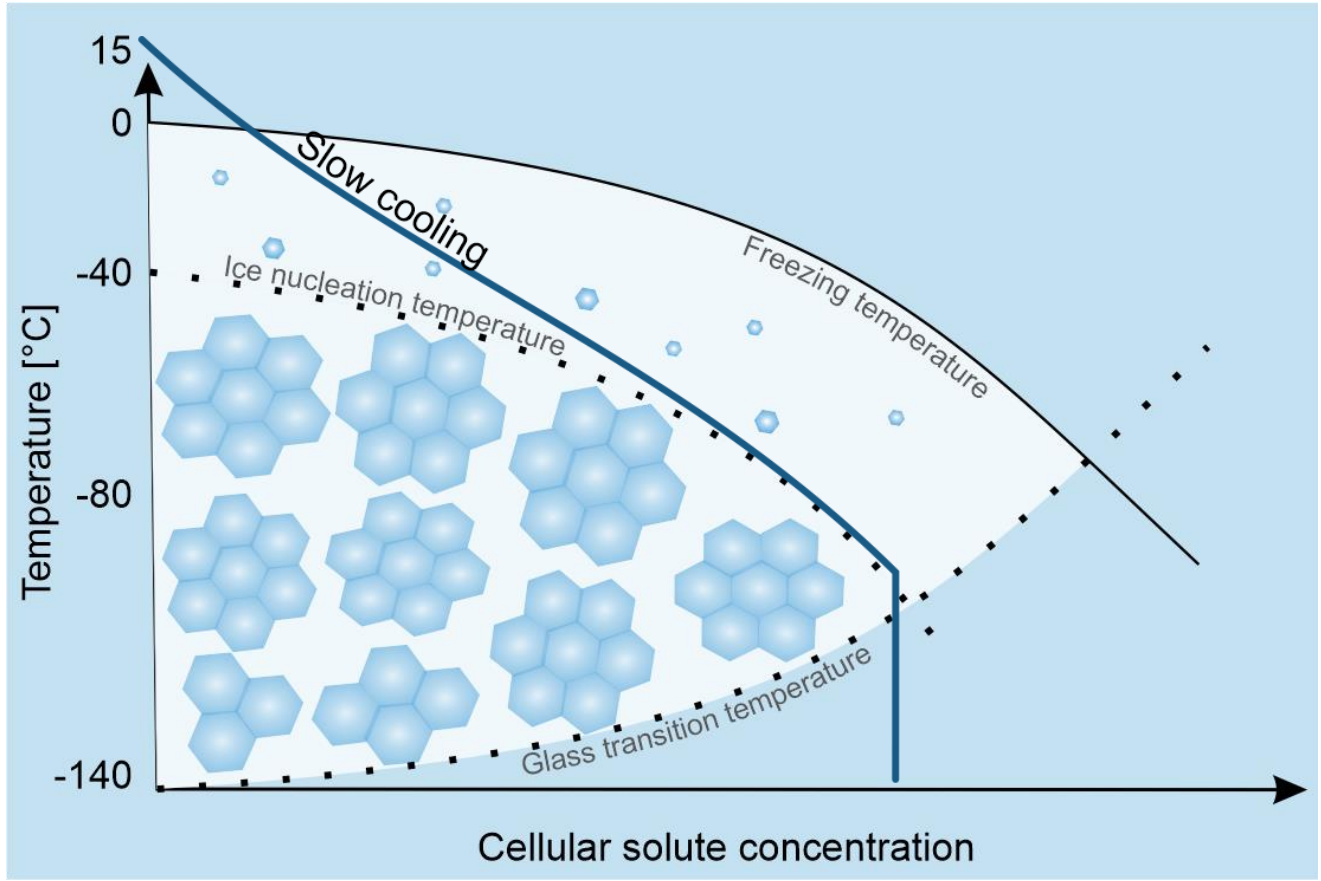
Water molecules

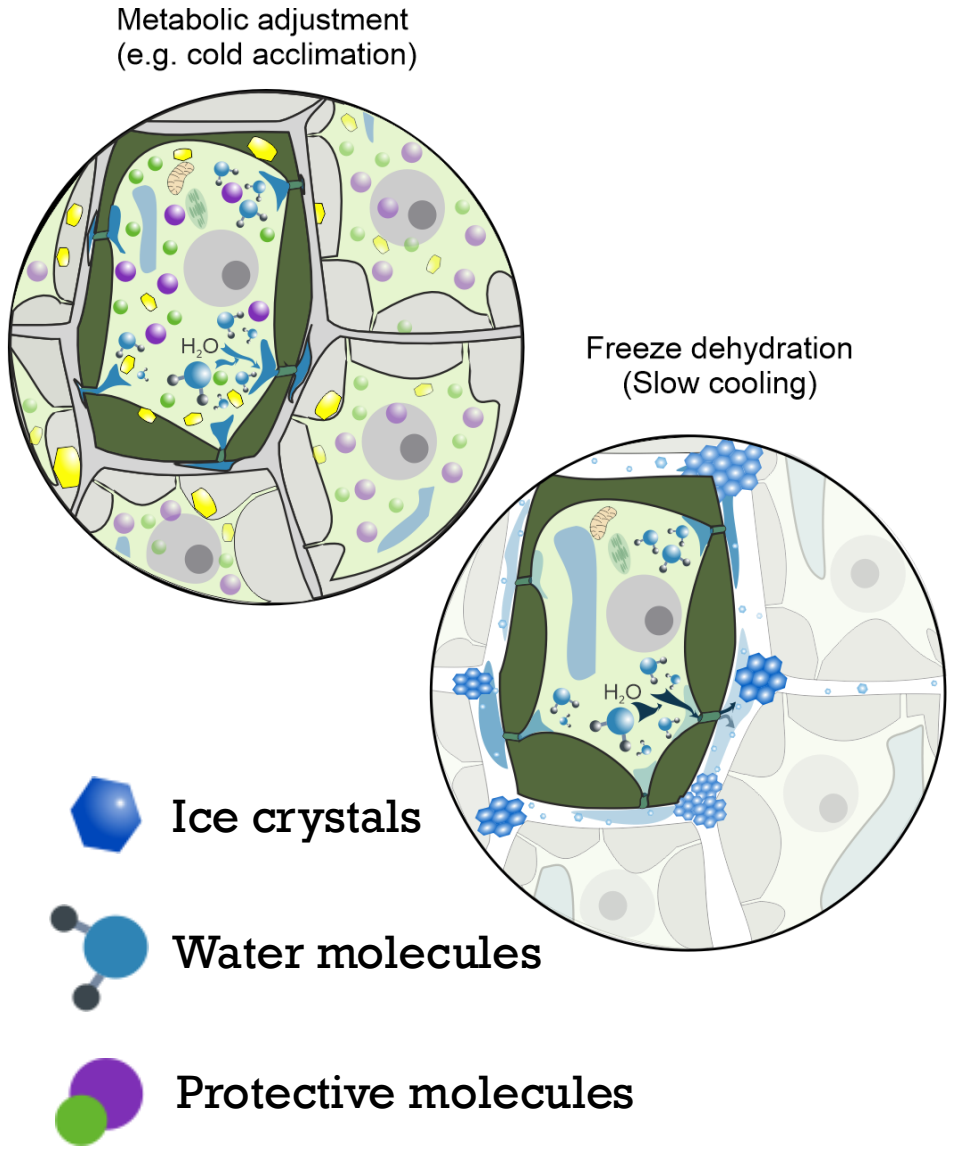
Orthodox seeds or pollen

Fast drying methods for embryonic axis of non-orthodox seed

Panis et al. (2001). https://doi.org/10.17660/ActaHortic.2001.560.8
Nagel et al. (2024) Annual Reviews of Plant Biology 75; 797-824
https://doi.org/10.1146/annurev-arplant-070623-103551

Cold acclimation & slow cooling

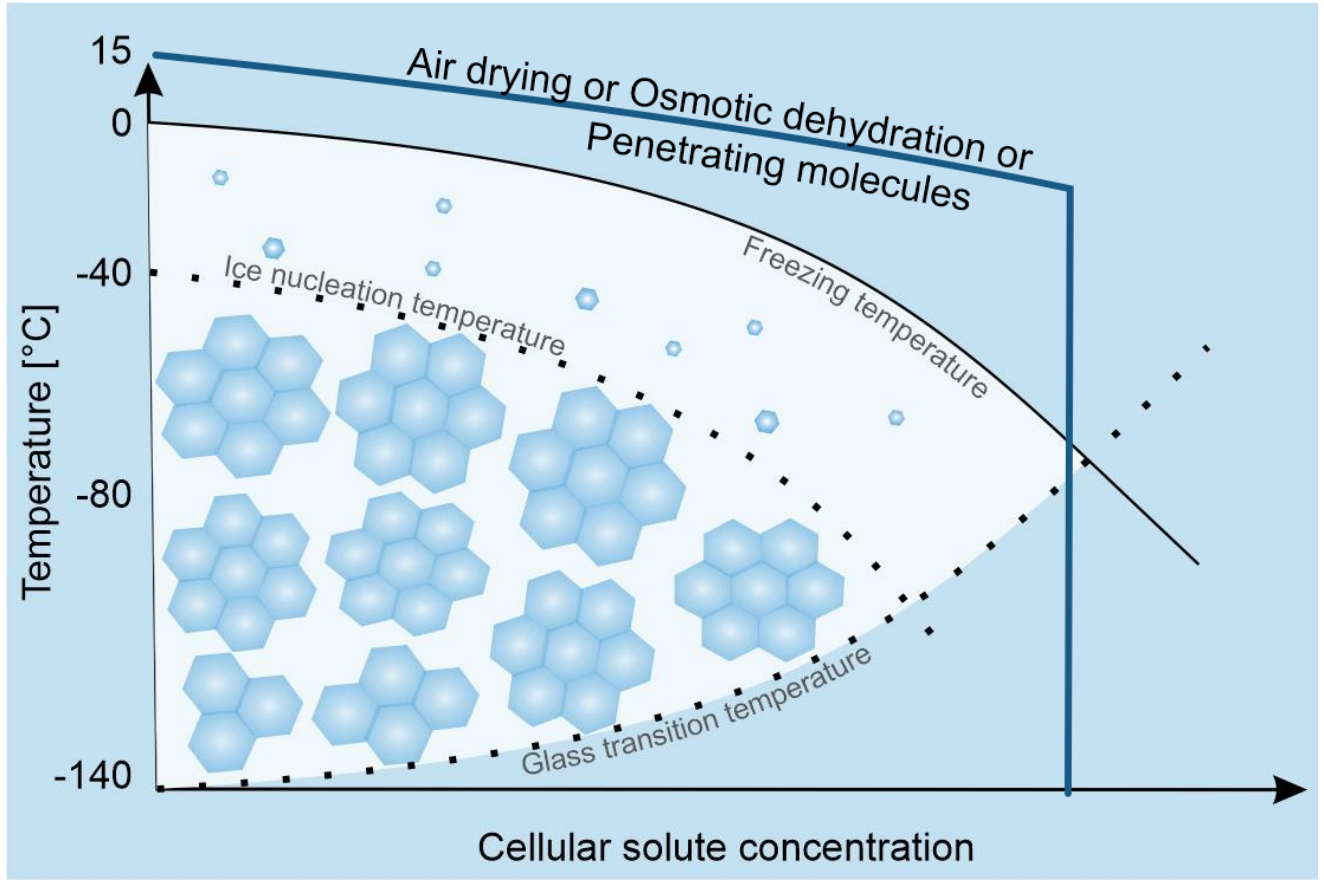


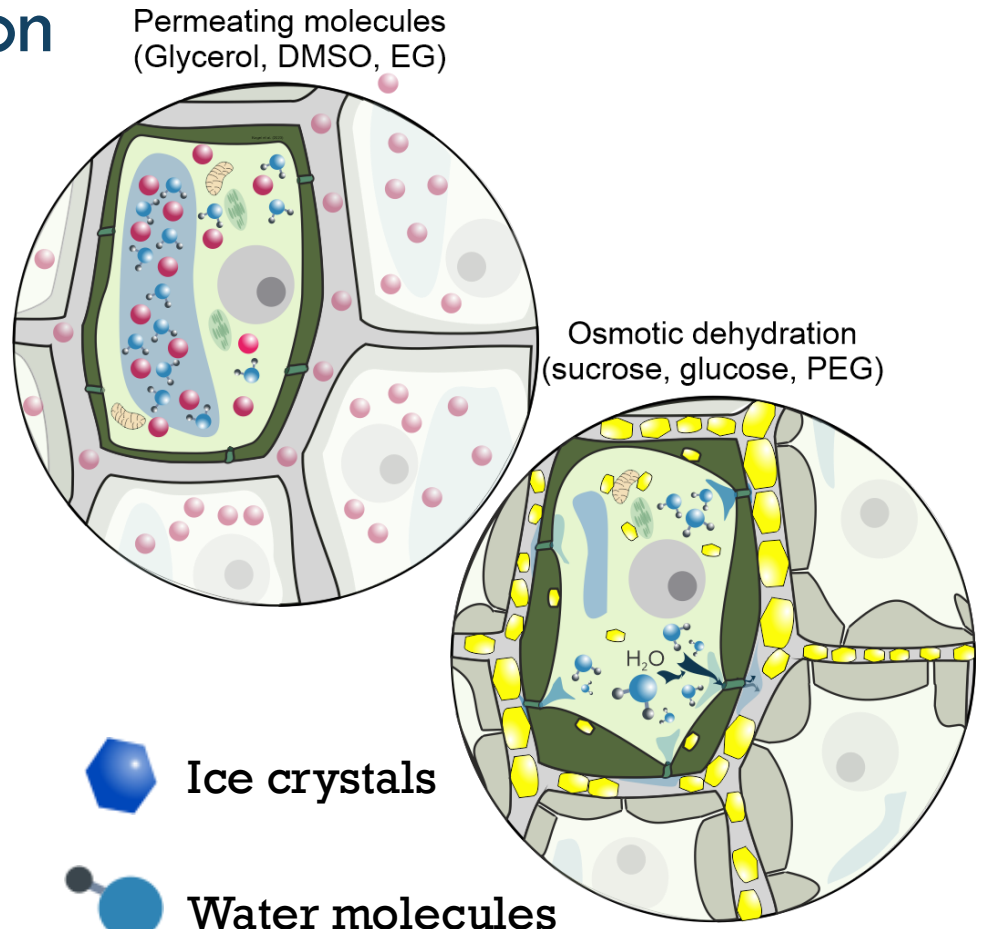


Dormant buds, unorganized cells, embryonic cells, cell lines

Panis et al. (2001). https://doi.org/10.17660/ActaHortic.2001.560.8
Nagel et al. (2024) Annual Reviews of Plant Biology 75; 797-824
https://doi.org/10.1146/annurev-arplant-070623-103551

Permeating molecules & osmotic dehydration





Ice crystals

Water molecules

Glycerol, DMSO

Sucrose or glucose

Shoot tips, embryonic axis of recalcitrant seeds

Panis et al. (2001). https://doi.org/10.17660/ActaHortic.2001.560.8
Nagel et al. (2024) Annual Reviews of Plant Biology 75; 797-824
https://doi.org/10.1146/annurev-arplant-070623-103551

Plant Cryoprotectant Agents

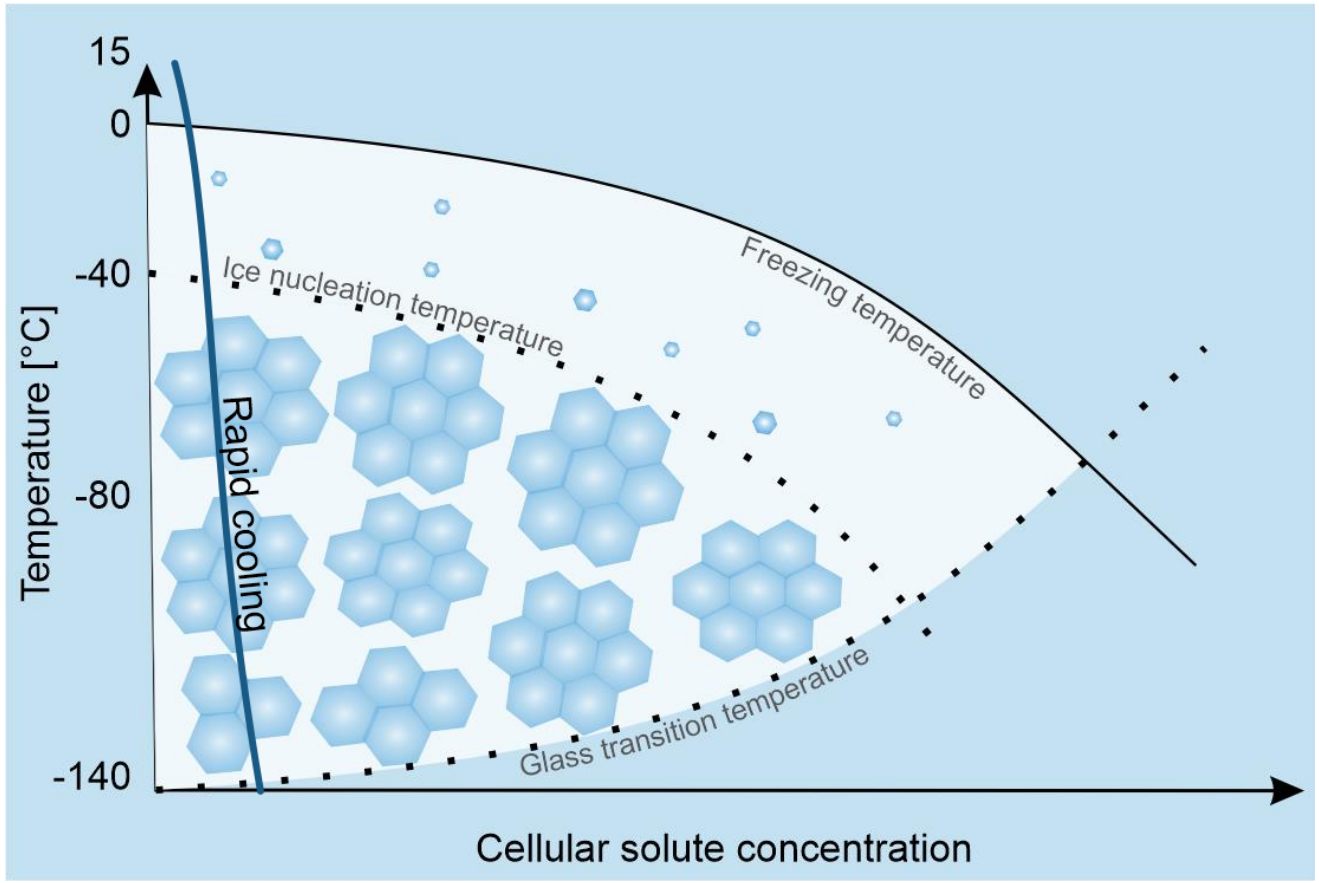
PVS 2 0.4 M Sucrose + 30% Glycerol + 15% Ethyleneglycol + 15% DMSO (Dimethylsulfoxide) (Sakai *et al*. 1990)

PVS 3 50% Sucrose + 50% Glycerol (Nishizawa *et al*. 1993)

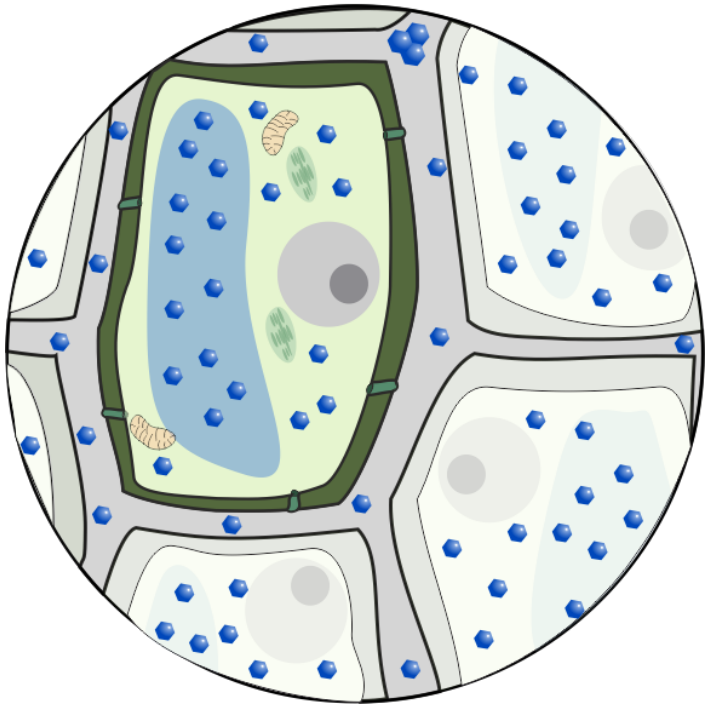
PVS 4 0.6 M Sucrose + 35% Glycerol + 20% Ethyleneglycol (Sakai 2000)

Droplet Freezing 10% DMSO (Schäfer-Menuhr *et al.* 1996)

Principles of conservation



Rapid cooling
(Without protection)



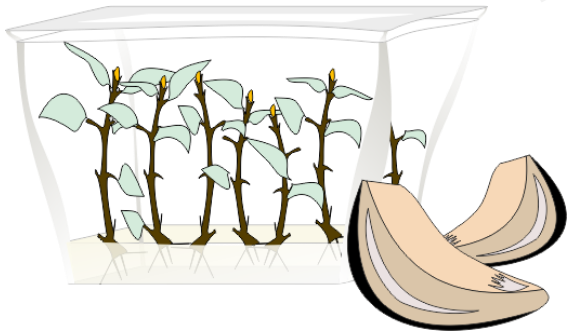
Ice crystals

Most non-vitrified tissue dies

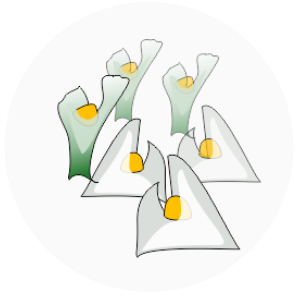
Panis et al. (2001). https://doi.org/10.17660/ActaHortic.2001.560.8
Nagel et al. (2024) Annual Reviews of Plant Biology 75; 797-824
https://doi.org/10.1146/annurev-arplant-070623-103551

How do we apply cryopreservation to potato and Allium species?

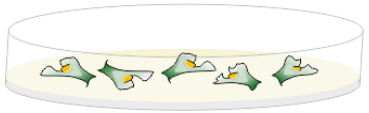
PVS3 droplet vitrification



Shoot tips from field progules or in vitro plants

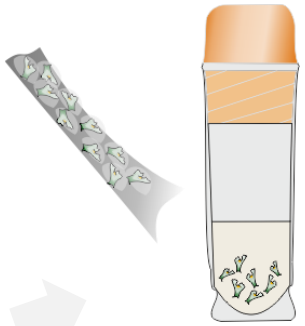


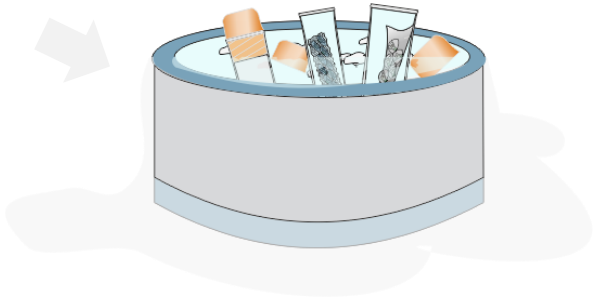
Excision & preculture



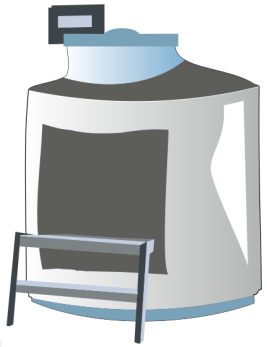
Loading & PVS3

Transfer to vials
Or into droplets

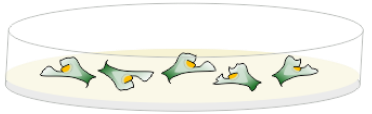




LN exposure

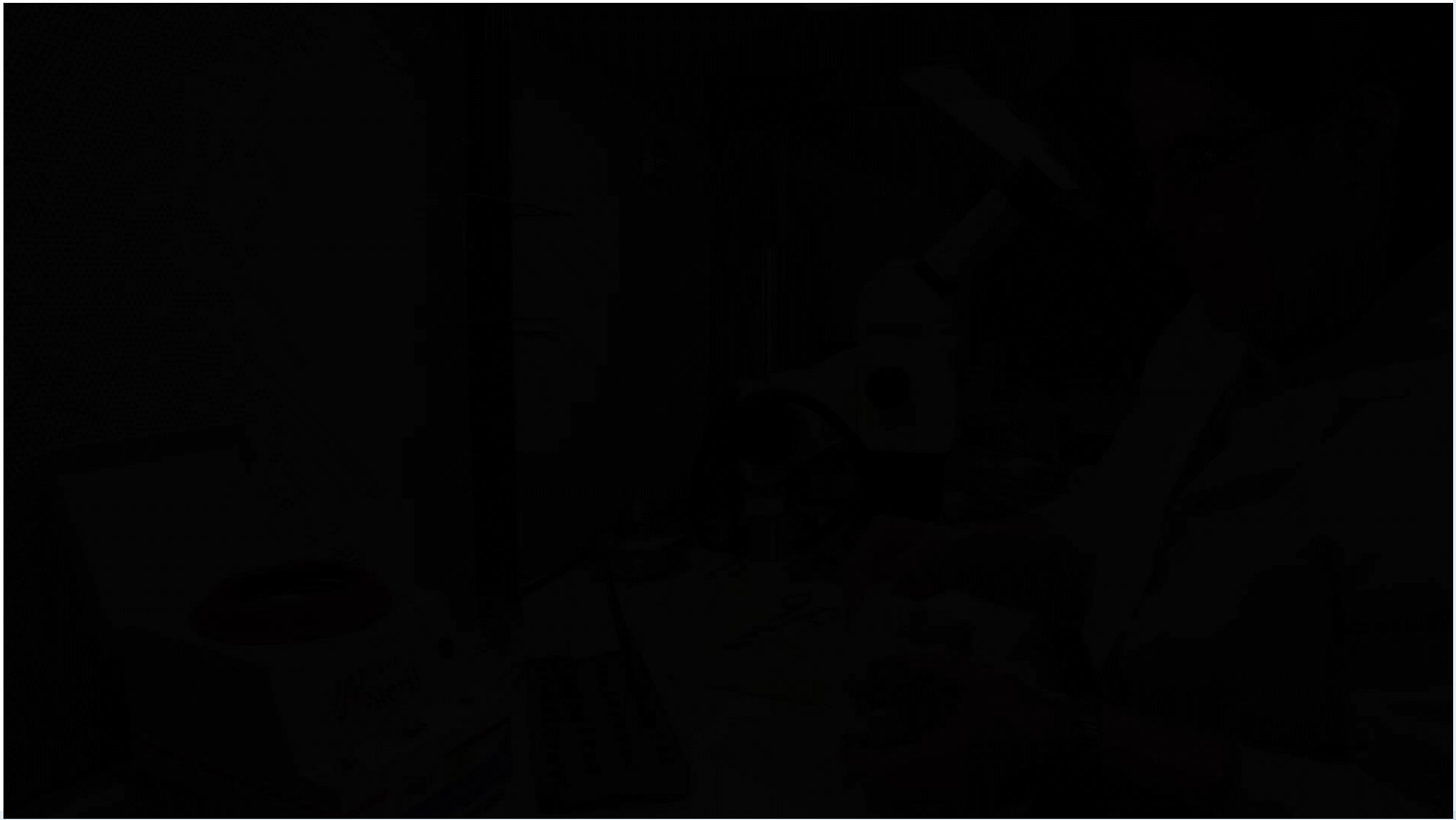


LN storage

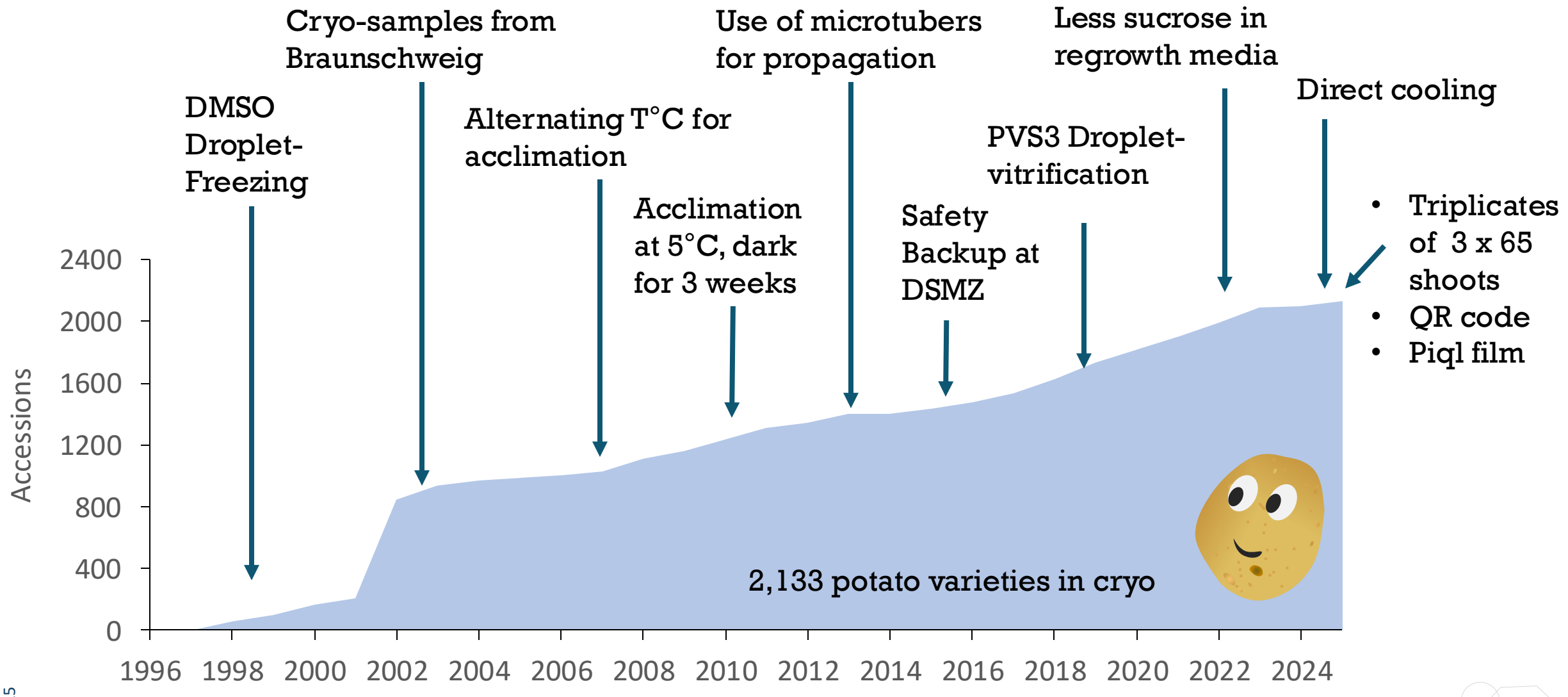


Rewarming

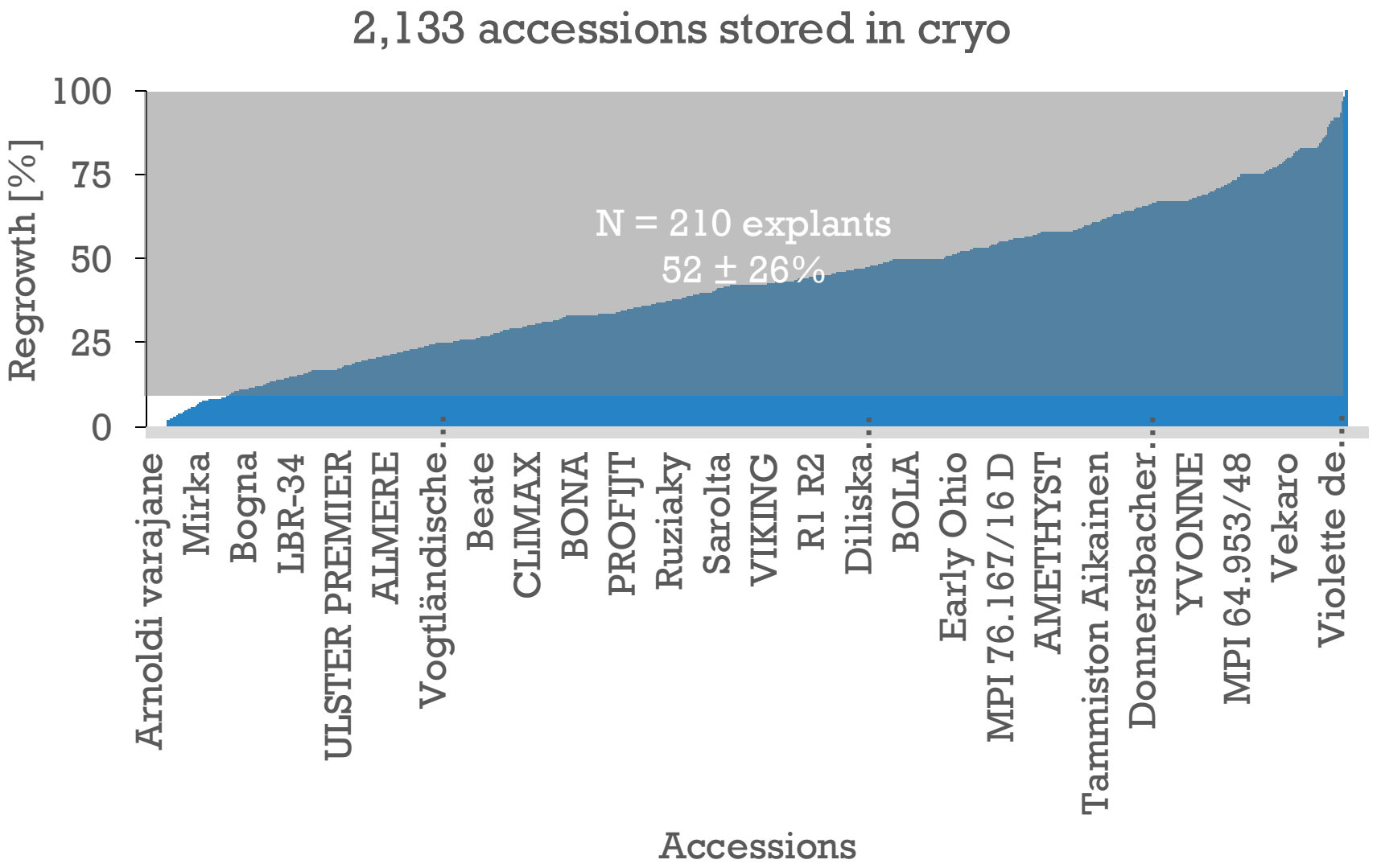
Nagel et al. (2024) Annual Reviews of Plant Biology 75; 797-824 https://doi.org/10.1146/annurev-arplant-070623-103551



Potato cryopreservation – adaptation during routine work

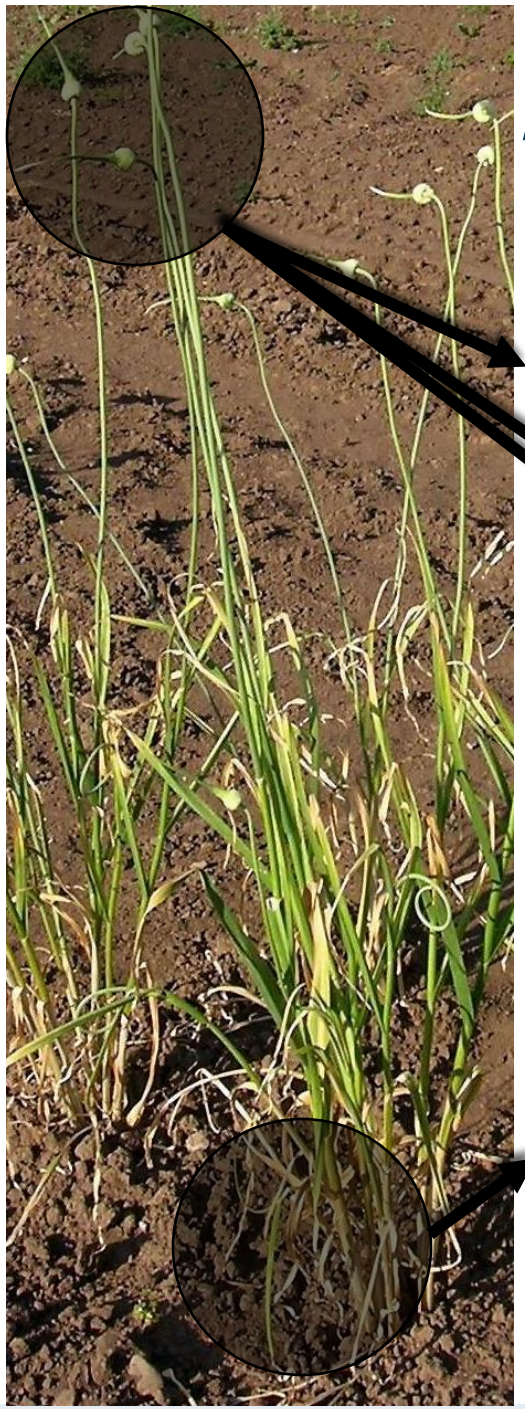


Regrowth varies strongly after cryopreservation



IPK, 2025, preliminary, unpublished data

Material for cryopreservation



Orthodox pollen

Immature inflorescences

Mature inflorescences/ bulbils

Cloves

Shoot tips

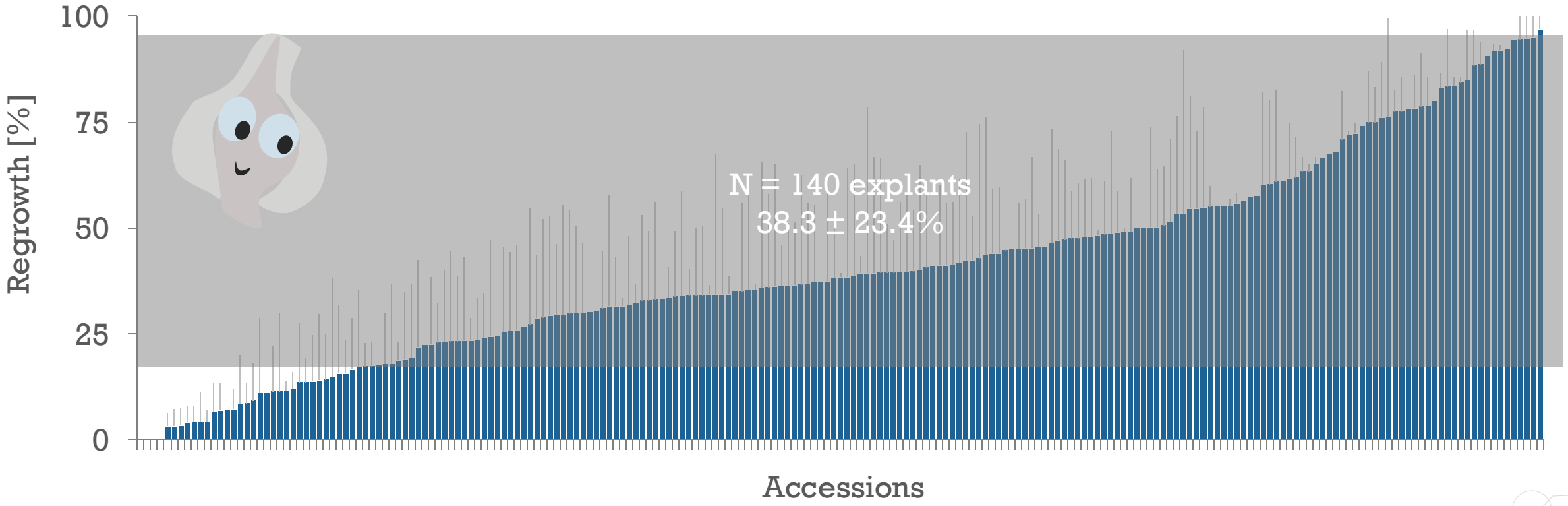
Meristems from basal plates

In vitro plants

Photo: E.R.J. Keller, Angelika Senula, Christine Zanke, H. Müller & M. Nagel

Regrowth varies strongly after cryopreservation

- Cloves, bulbils, *in vitro* plants and inflorescences
- Cryopreservation conducted on 224 accessions



IPK, 2025, preliminary, unpublished data

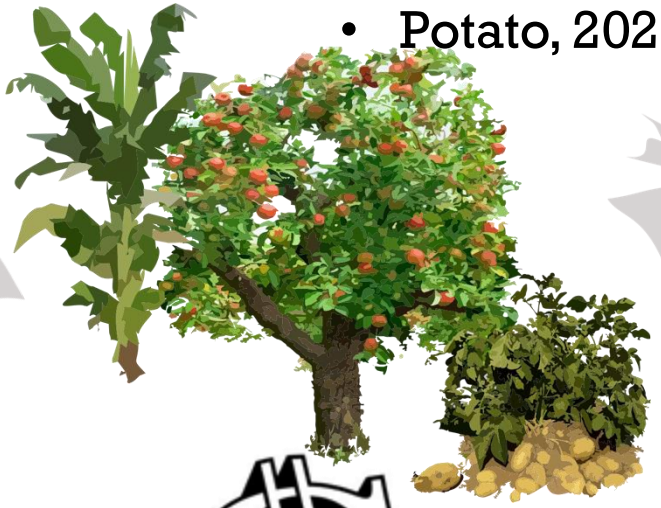
What are the challenges of cryopreservation?

Costs

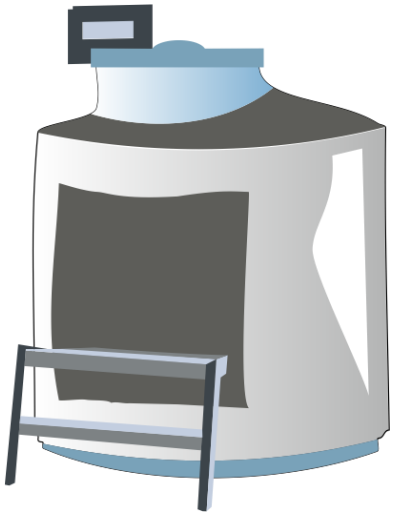
Cost of cryopreservation

- Garlic, 2013: 47 EUR Field, 360-560 EUR Cryo
- Banana, 2020: 900 USD In-vitro, 1.300 USD Cryo
- Potato, 2025: 3.000-5.000 EUR

Field genebanks
~400,000 accessions



In vitro genebanks
~33,000 accessions



Cryobanks
~17,690 accessions

- Well-equipped in vitro labs
- Trained personnel

Nagel et al. (2024) Annual Reviews of Plant Biology 75, 797-824 https://doi.org/10.1146/annurev-arplant-070623-103551
Panis et al. (2020) Plants 9; https://doi.org/10.3390/plants9121634

Duplication status

10-15% potential duplication

ALL 651, 1981, Bagnoli, Italy

ALL 1840: Italian Purple, 1986, USA

ALL 651

ALL 1840

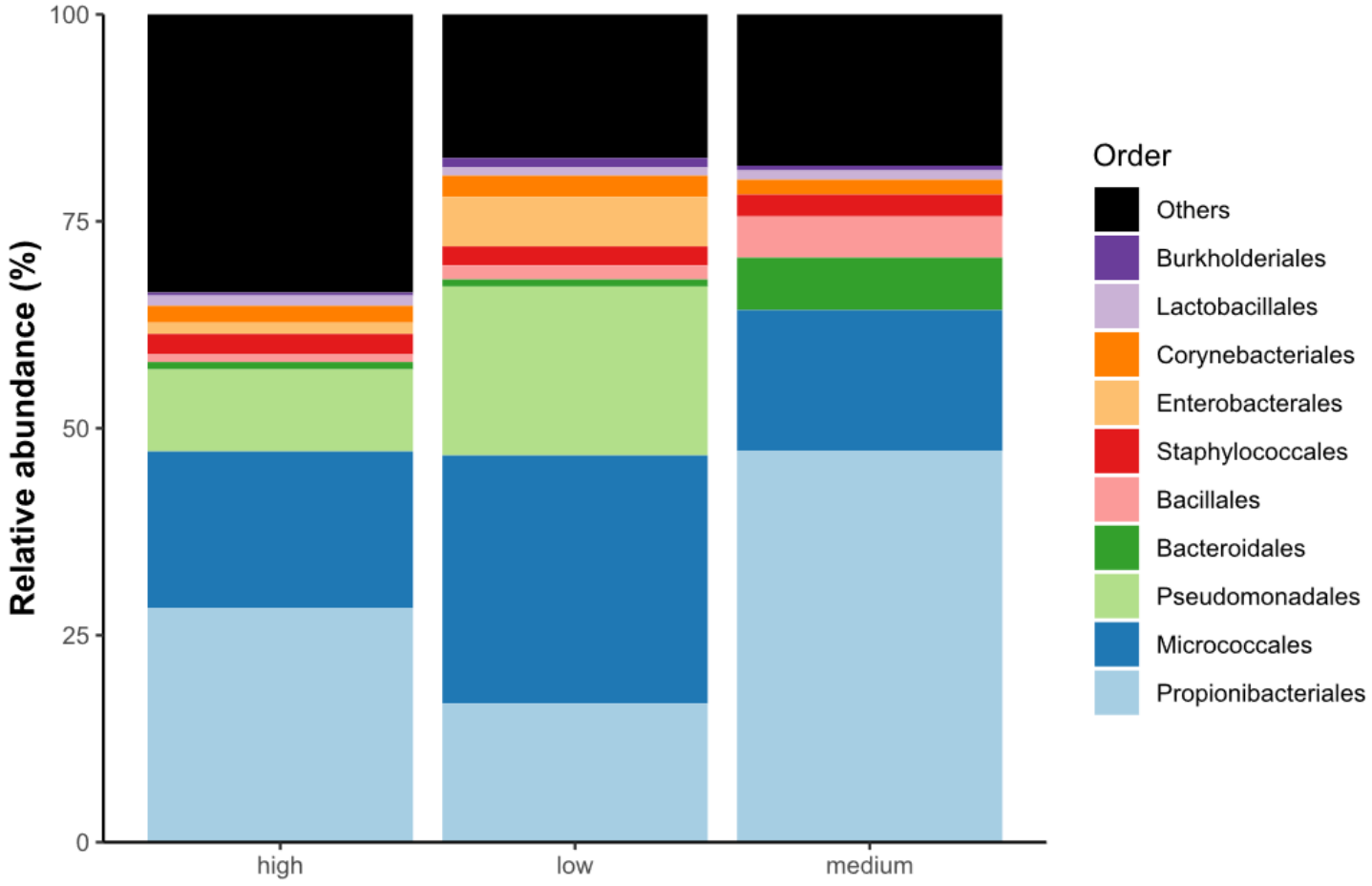
IPK, 2025, preliminary, unpublished data

3

Contaminations and endophytic organisms

Microbiome of potato accessions

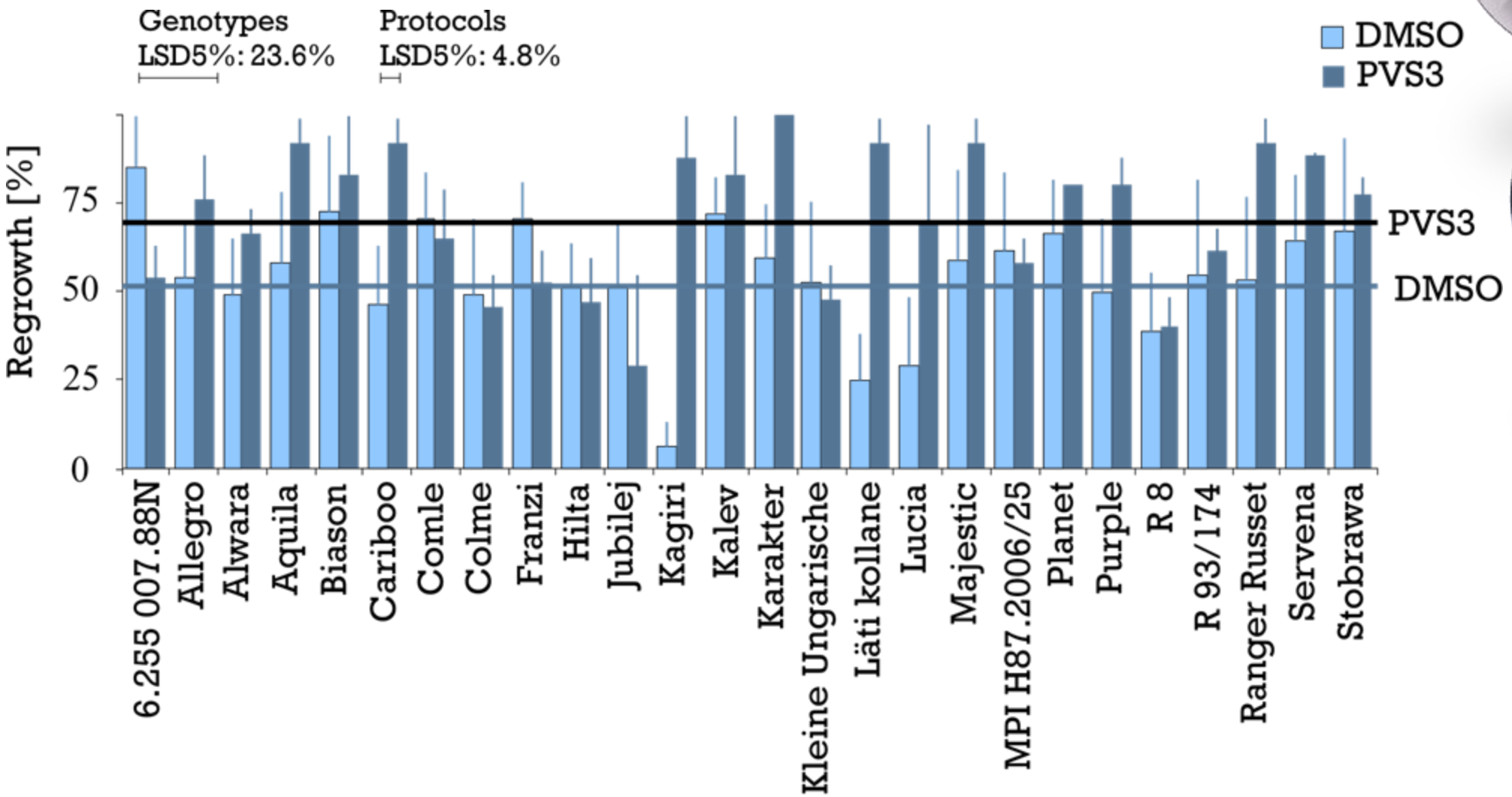
- Barcoding of 384 potato accessions with high and low regrowth



IPK, 2025, preliminary, unpublished data

Protocol development

DMSO vs. PVS3



Köpnick et al. (2018) https://doi.org/10.1016/j.cryobiol.2018.09.005

DMSO vs. PVS3 vs PVS3A

Köpnick et al. (2018) https://doi.org/10.1016/j.cryobiol.2018.09.005

DMSO Droplet Freezing

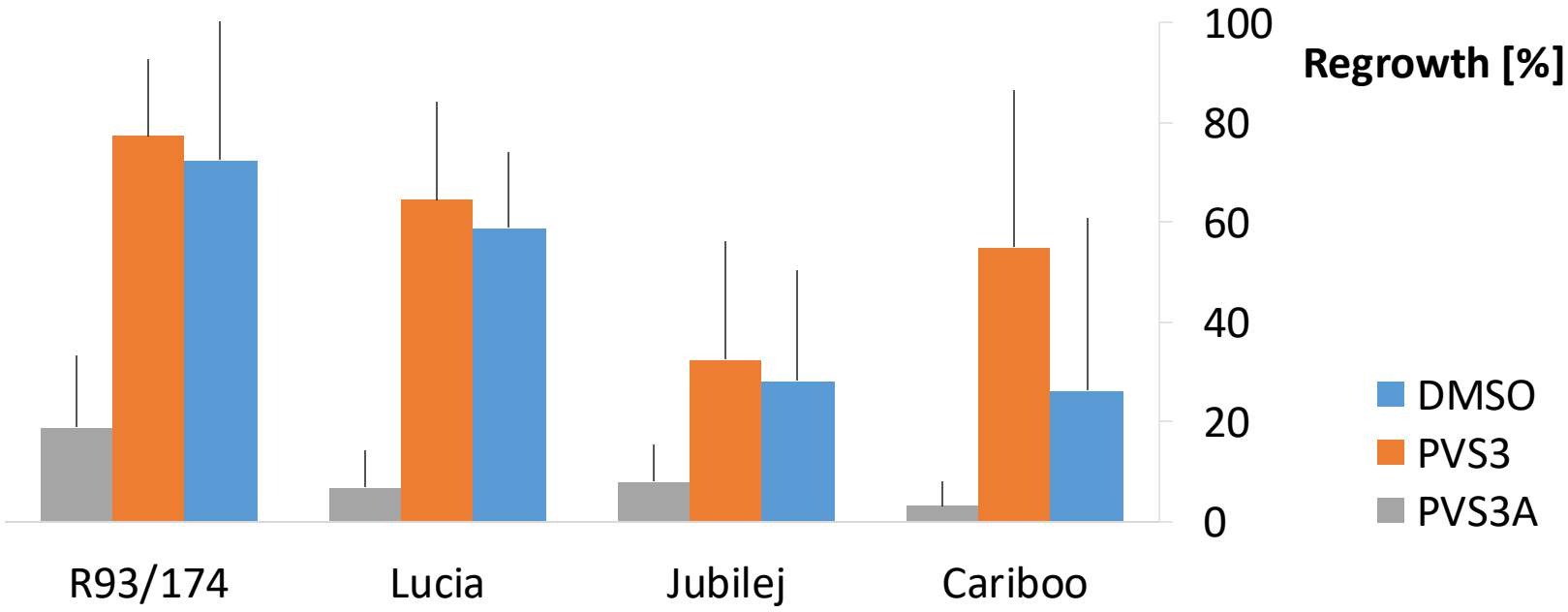
20 h Preculture

PVS3 Droplet Vitrification

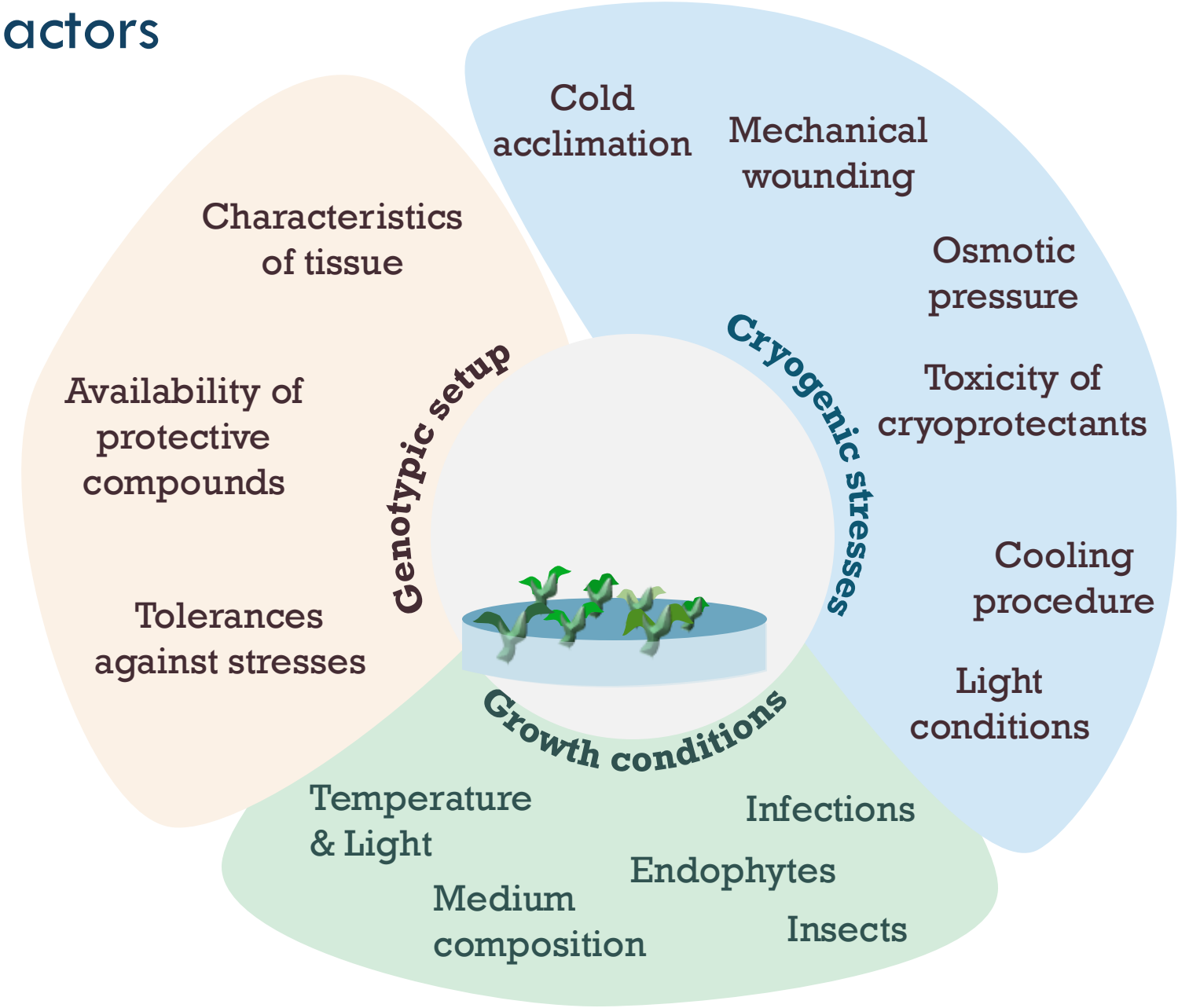
20 h Preculture

PVS3A Droplet Vitrification

30 min Preculture



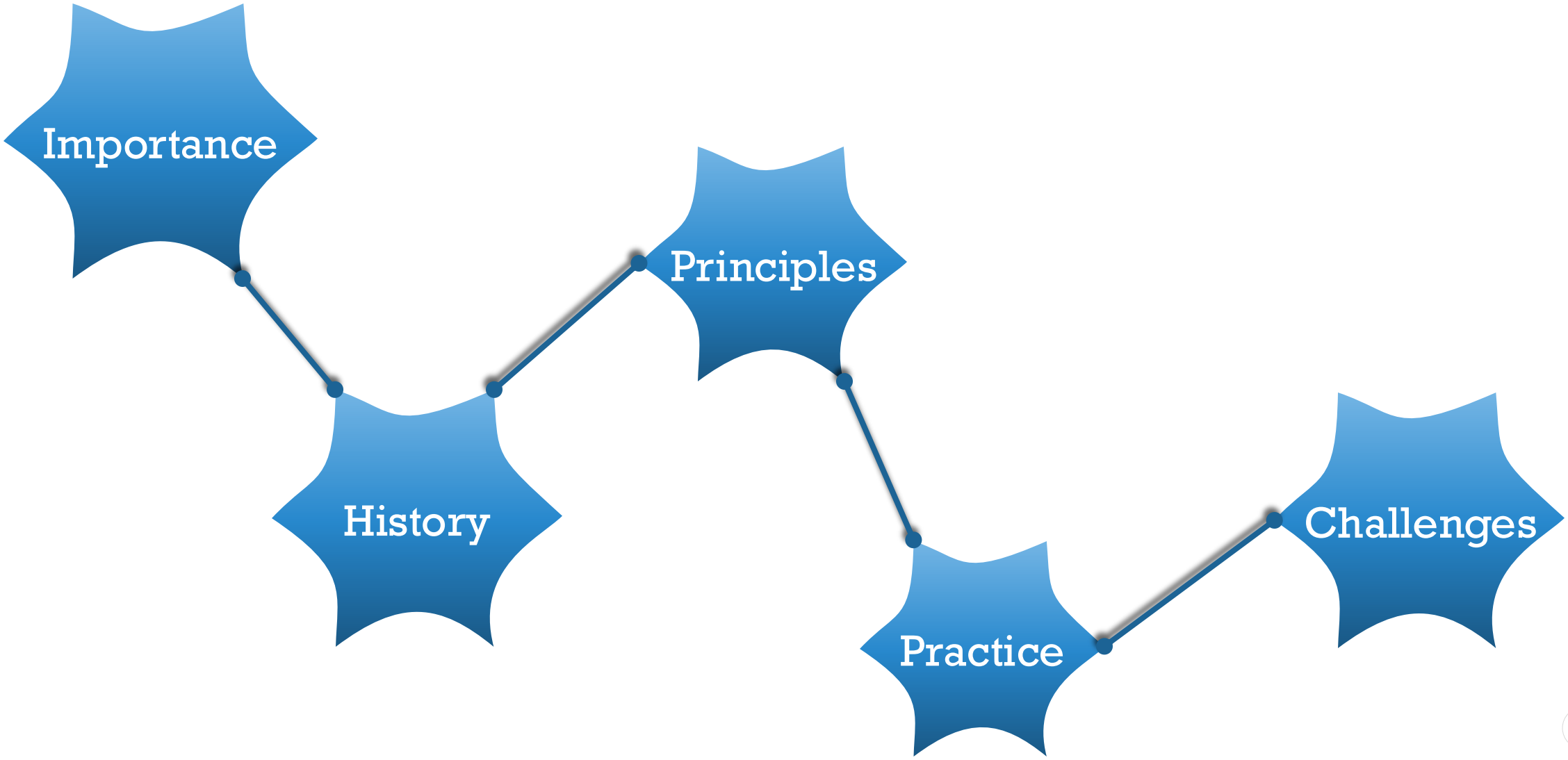
Influencing factors



Further challenges

- Cryopreservation is slow
- Epigenetic effects needs to be furhter investigated
- Standardization and data security
- Back-up storage and storage security
- Cryopreservation of PGR and wild species can be only successful as a complementary approach

Structure



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Thank you for your attention!

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