Ex situ techniques: How to implement them, what are the skills in the Mediterranean

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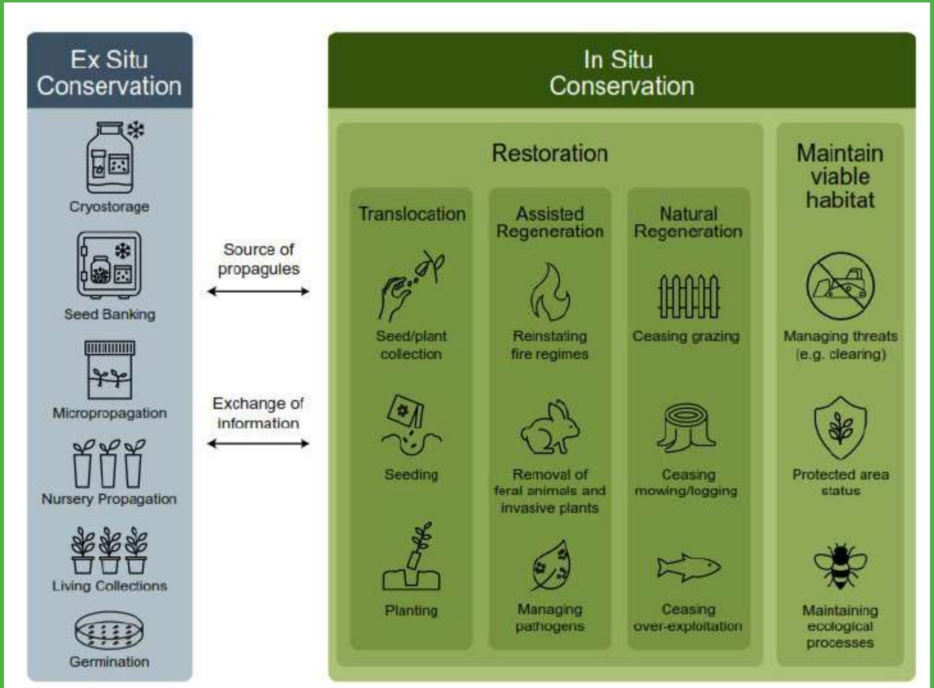
EX SITU CONSERVATION

Global Strategy for Plant Conservation (GSPC)

- ✓ Target 8 of the GSPC (2011–20): At least 75% of threatened plant species in ex situ collections, preferably in the country of origin, and at least 20 % available for recovery and restoration programmes.
- ✓ Suggested Plant Conservation Objectives for 2050 (Development of a post-2020 GSPC, CBD, Feb2021):
    - All known threatened wild plant species are effectively conserved and managed in situ and **ex situ**, including viable populations.
    - All socio-economically important plant species, including crop wild relatives, are effectively conserved and managed in situ and **ex situ**.

Connecting in situ with ex situ conservation



Commander LE (Ed.) (2021) 'Florabank Guidelines – best practice guidelines for native seed collection and use (2nd edn).' (Florabank Consortium: Australia)

SEED BANKING

storage of dessicated seeds at –20 ℃

1

TISSUE CULTURE

growth of tissues or cells in an artificial medium

2

3

storage of living material at very low temperatures

CRYOPRESERVATION

4

cultivation of plants in botanical gardens

LIVING PLANT COLLECTIONS

1.
Seed Banking

Genmeda
NETWORK OF MEDITERRANEAN PLANT CONSERVATION CENTRES

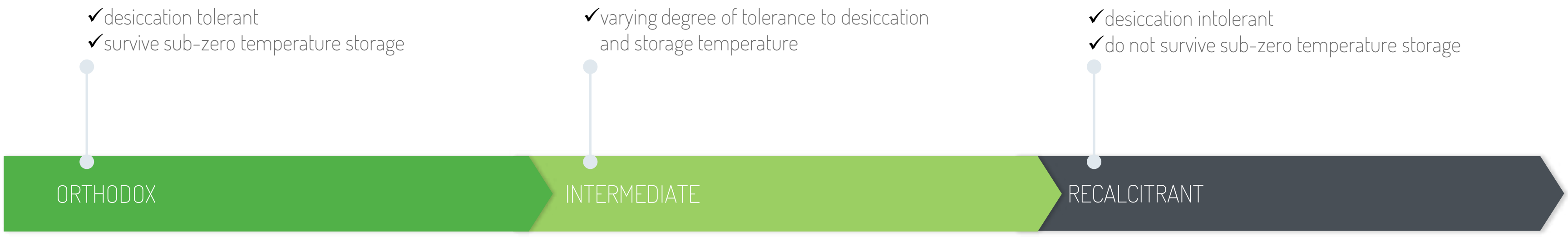
ENSCONET
European Native Seed Conservation Network

RIBES – Italian network of seedbanks

REDBAG – Spanish Network of genebanks for wild plants


ENSCONET
European Native Seed Conservation network
ENSCONET
Seed Collecting Manual
FOR WILD SPECIES
Main editors:
Royal Botanic Gardens, Kew (UK) &
Universidad Politécnica de Madrid (Spain)
Edition 1: 17 March 2009*
* This document will be updated as improvements become appare
ISBN: 978-84-692-3926-1
Citation: ENSCONET (2009) ENSCONET Seed Collecting Manual for Wild
ENSCONET
Curation Protocols
& Recommendations
Overall editor:
Royal Botanic Gardens, Kew
Version: 15 June 2009
ISBN: 978-84-692-5964-1
Citation: ENSCONET (2009) ENSCONET Curation Protocols & Recomm
ENSCONET
Germination Recommendations
UPDATED
Overall editor:
Royal Botanic Gardens, Kew
Version: 28 October 2009
Citation: ENSCONET (2009) ENSCONET Germination Recommendations - Updated
APAT
Manual for the collection, study, ex situ processing and conservation of germplasm
Manuals and Guidelines 37/2006
Legal Information
juin 2004 – mai 2006
MEDOCC
GenMedOc
Pratiques de germination dans les banques de semences du réseau GENMEDOC
juin 2004 – mai 2006
1

Seed storage behavior



Variability in seed storage behaviour between species, rather than three discrete categories, is more appropriately viewed as a continuum with respect to the degree of seed desiccation tolerance and the impacts and interactions of seed water content and storage temperature on longevity

Walters C.2015. Orthodoxy, recalcitrance and in-between: describing variation in seed storage characteristics using threshold responses to water loss. Planta242: 397-406

Storage conditions for seeds banked for different time frames and for different purposes

Time frame	Conditions	Suitable for:
Short term (≤ 5 years)	Air-conditioned room c. 23 °C, or refrigerator or cool room (5–15 °C) Ambient relative humidity if <50 %	Restoration seed banks
Medium term (≤ 10 years)	Temperature 5–10 °C Relative humidity 15–20 % Seed moisture content c. 3–7 %	**Active collections**, including those for plant breeding and research
Long term (>10 years)	Temperature ≤ minus 18 °C Relative humidity 15–20 % Seed moisture content c. 3–7 %	Conservation seed banks including wild species and agricultural genebanks; **base collections**.

Yenson M AJ et al. 2021. Plant Germplasm Conservation in Australia: strategies and guidelines for developing, managing and utilising ex situ collections. Third edition. Australian Network for Plant Conservation, Canberra.

Pros & Cons of Seed Banking

PROS

- ✓ long term storage
- ✓ relatively low cost
- ✓ appropriate method for the majority of plant species

CONS

- ✓ not suitable for recalcitrant species
- ✓ storage/germination protocols yet to be developed
- ✓ seed ageing

The most efficient and widely-used method of ex situ conservation.

The best current estimate of the presence of desiccation tolerance vs desiccation sensitivity amongst species is c. 90 % and c. 8 %, respectively

Wyse, S. and J. Dickie. "Predicting the global incidence of seed desiccation sensitivity." Journal of Ecology 105 (2017): 1082-1093.

Exceptional Species

plant species that cannot be efficiently and effectively conserved long-term ex situ under the conditions of conventional seed banking, requiring alternative conservation approaches

Pence VC et al. 2020. Cryobiotechnologies: Tools for expanding long-term ex situ conservation to all plant species. Biological Conservation 250, 1-8.

Definition

- do not produce seed
- are extremely difficult to harvest
- do not survive conventional seed bank conditions
- do not remain viable for a reasonable length of time in seed bank conditions
- do not germinate and do not produce plants upon removal from a seed bank even though they are viable

- Projection: >30,000 spp.
- Examples : oaks, alpine species, orchids
- Compilation of a global list of threatened exceptional species

2.
Tissue culture

Plant tissue culture is defined as culturing plant seeds, organs, explants, tissues, cells, or protoplasts on a chemically defined synthetic nutrient media under sterile and controlled conditions of light, temperature, and humidity.

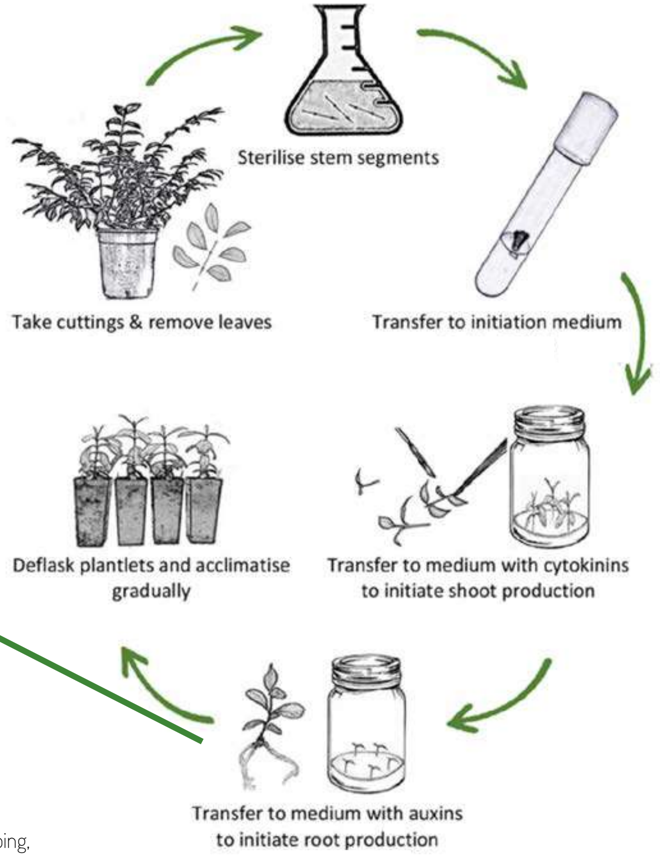
Bhatia S et al. 2015. Chapter 1 - History and Scope of Plant BiotechnologyModern Applications of Plant Biotechnology in Pharmaceutical Sciences, Academic Press, Pages 1-30

Main tissue culture systems

- Micropropagation
- Callus culture
- Suspension cell culture
- Somatic embryogenesis

Key steps in micropropagation

Slow growth storage



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Pros & Cons of Tissue Culture

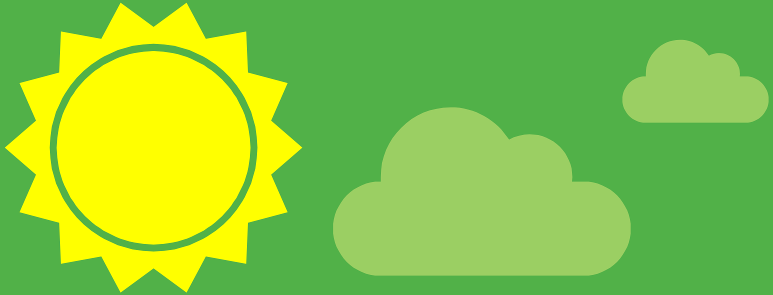
PROS

- ✓ high volume plant production
- ✓ plants protected against environmental hazards
- ✓ large number of plants in limited space

CONS

- ✓ specialized expertise
- ✓ low genetic variability
- ✓ development of protocols for new taxa – expensive / time consuming
- ✓ possible genetic changes
- ✓ contamination risk
- ✓ difficult transition from culture

https://www.rbgsyd.nsw.gov.au/Science/Australian-PlantBank-1/Conservation-in-action/Tissue-culture-and-cryopreservation/Cryopreservation



Why choose tissue culture

- large-scale micropropagation of plants
- conserve highly threatened species
- reproduce plants that don't produce seeds or don't germinate well
- conserve plants not suited to seedbanking
- prepare material for cryopreservation

Adapted by https://www.rbgsyd.nsw.gov.au/Science/Australian-PlantBank-1/Conservation-in-action/Tissue-culture-and-cryopreservation/Tissue-culture

Case study: *Asparagus macrorrhizus*

- ✓ developed protocol allowed the micro-propagation of plantlets true-to-type
- ✓ establishment of an in vitro *Asparagus macrorrhizus* germplasm bank
- ✓ Institute of Hortofruticulture Subtropical and Mediterranean “La Mayora” (IHSM, Malaga, Spain).

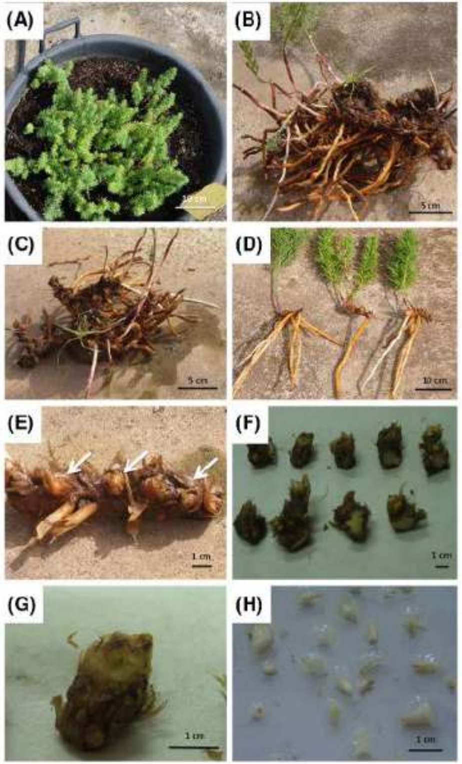


Fig. 1 Procedure for extraction of rhizome bud explants from *Asparagus macrorrhizus*: **a** mother plants, **b–d** morphology of plant rhizomes, **e–g** details of rhizome bud clusters, **h** dissected rhizome bud explants

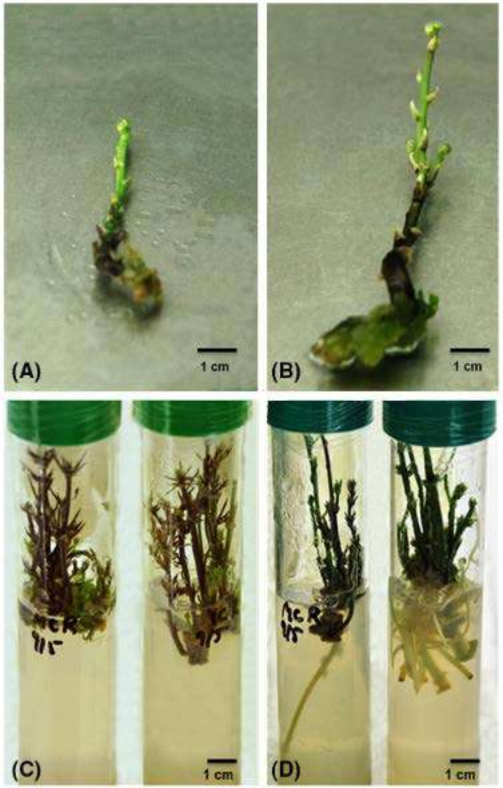


Fig. 2 *A. macrorrhizus* micropropagation. **a** and **b** Rhizome bud explants cultured on ARBM for 4 weeks. **c** Shoots obtained from rhizome bud explants. **d** Shoots rooted in MRM-2

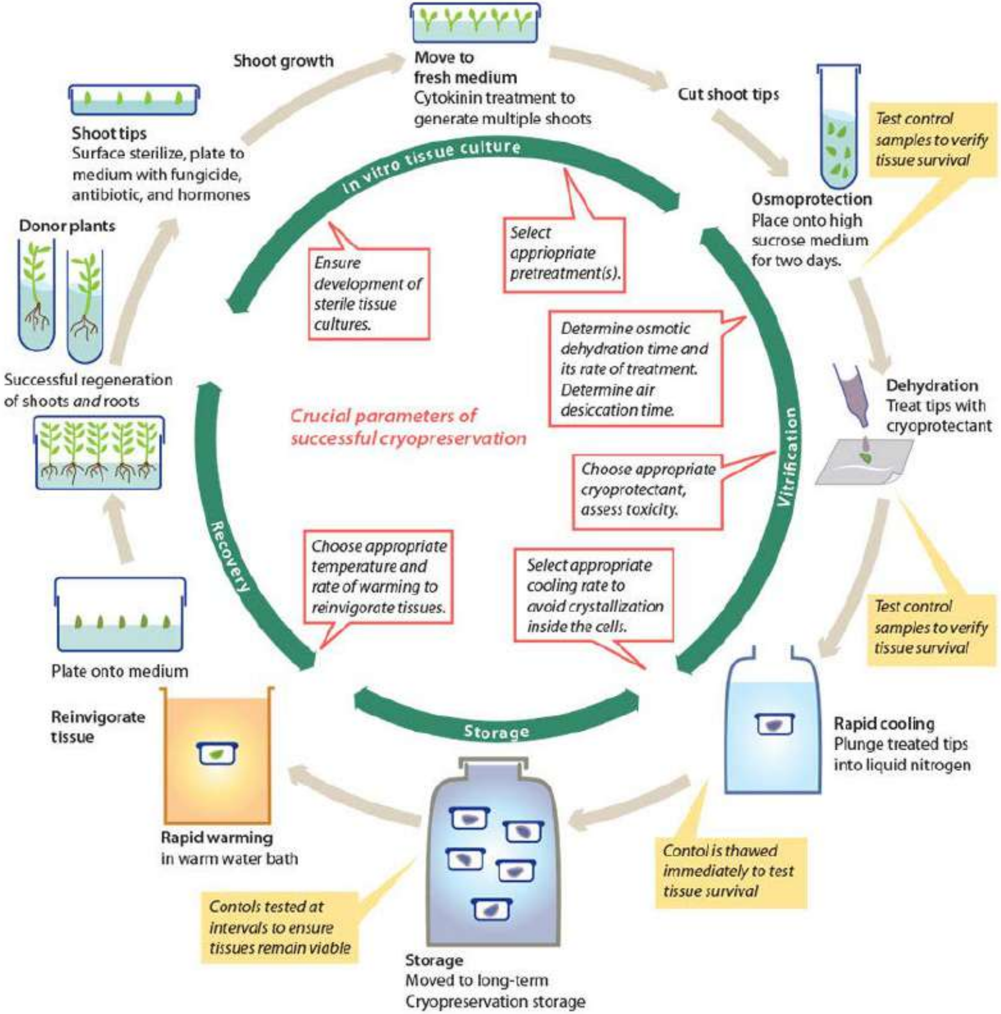
Regalado, J.J. et al. 2018. Micropropagation of Asparagus macrorrhizus, a Spanish endemic species in extreme extinction risk. Plant Cell Tissue Organ Cult., 132, 573–578.

3. Cryopreservation

Cryopreservation is the storage of living material at very low temperatures, typically utilising liquid nitrogen (-196°C) or its vapour (-130 to -192°C).

What to cryopreserve

- Short-lived Seeds
- Zygotic Embryos
- Dormant Buds
- Somatic Embryos
- Shoot Tips
- Pollen



https://saveplants.org/best-practices/collecting-and-maintaining-exceptional-species-tissue-culture-and-cryopreservation/

Pros & Cons of Crypreservation

PROS

- ✓ relatively low cost
- ✓ minimal storage space
- ✓ low maintenance workload
- ✓ minimum contamination risk

CONS

- ✓ specialized expertise
- ✓ development of protocols for new taxa – expensive / time consuming
- ✓ regular liquid nitrogen supply

Each plant species and tissue type has very specific requirements for the way in which it is prepared, frozen and thawed. So each species and tissue combination has to be tested individually before cryopreservation can be successfully applied to many plants.

https://www.rbgsyd.nsw.gov.au/Science/Australian-PlantBank-1/Conservation-in-action/Tissue-culture-and-cryopreservation/Cryopreservation

Case study: *Quercus robur*

- ✓ developed protocol allowed the cryopreservation of somatic embryos
- ✓ pre-culture on 0.3 M sucrose medium followed by application of PVS2 solution for 60–90 min prior to being cooled in liquid nitrogen
- ✓ Instituto de Investigaciones Agrobiologicas (Galicia, Spain).

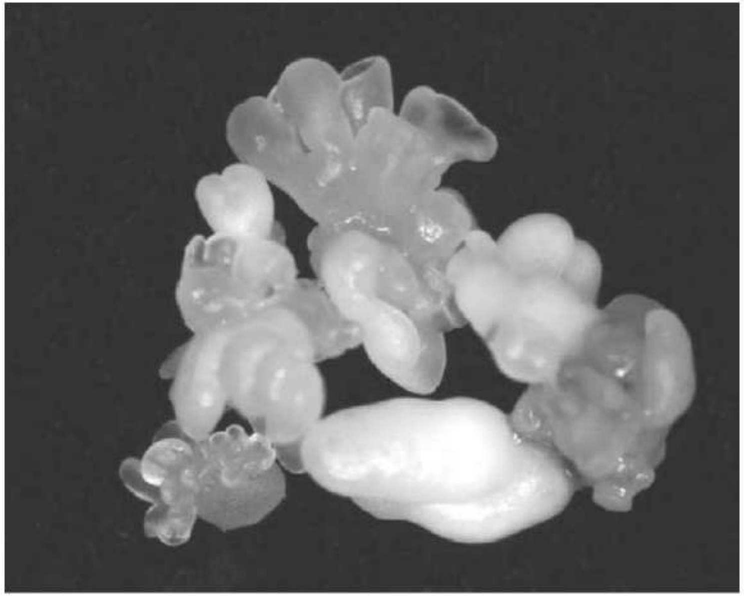


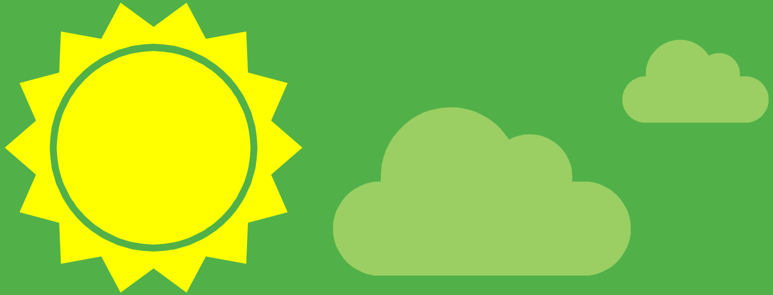
Fig. 1. Proliferation of *Q. robur* somatic embryos (line T4-H) frozen in liquid nitrogen following pre-culture on high-sucrose medium and dehydration treatment.

M.T. Martínez, et al. 2003 . Cryopreservation of embryogenic cultures of Quercus robur using desiccation and vitrification procedures, Cryobiology, Volume 46, Issue 2, Pages 182-189,.

4.
Living plant collections

Types of living collections

1. Botanic gardens, arboreta and specialist horticultural gardens
2. Field genebanks



Why choose living plant collections

- conserve exceptional species
- ensure clonal replicates where unique or elite genotypes need conservation
- conserve threatened, easily cultivated species
- produce material for restoration
- produce material for other ex situ techniques
- supply material in order to remove or reduce pressure on wild populations
- provide parent material for field genebanks

Adapted from Yenson M AJ et al. 2021. Plant Germplasm Conservation in Australia: strategies and guidelines for developing, managing and utilising ex situ collections. Third edition. Australian Network for Plant Conservation, Canberra.

Important to keep in mind

- conservation value is largely determined by the extent of genetic representativeness of the species
- use of living collections in combination with other conservation techniques
- in certain cases, and particularly species with very small populations, it may be the only practical conservation option available

Pros & Cons of Living Plant Collections

PROS

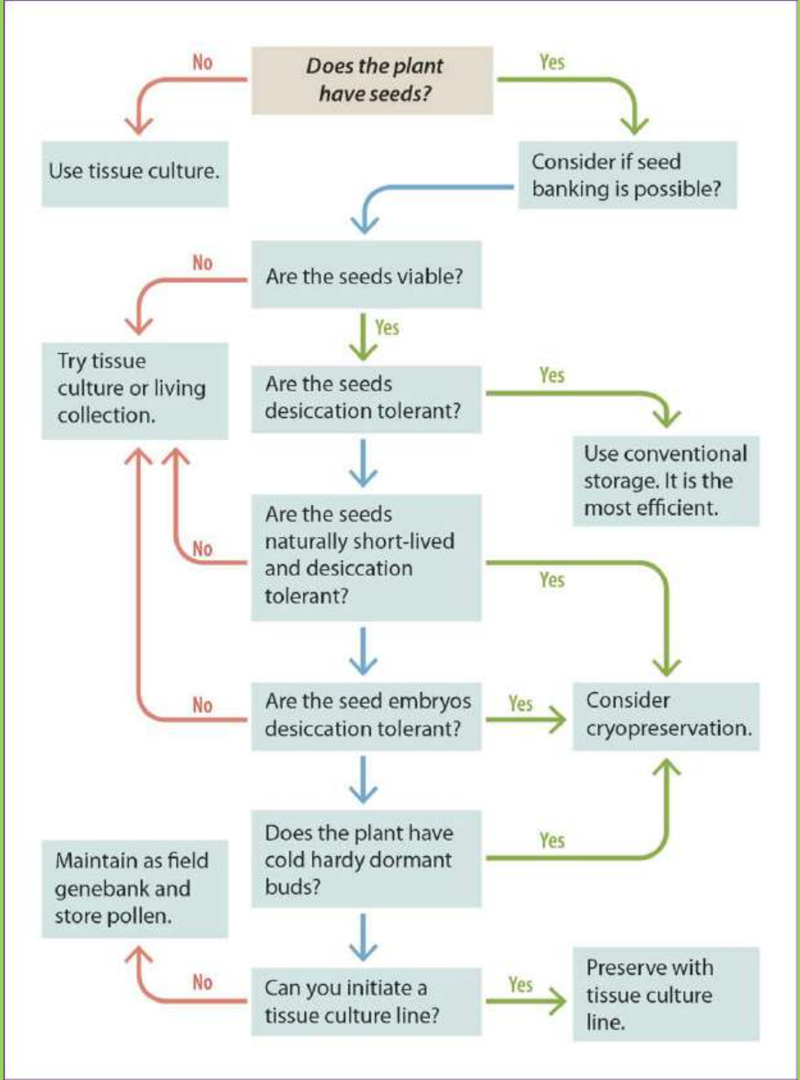
- ✓ ready access material for trasplantation
- ✓ constant supply of seed or vegetative material
- ✓ increase knowledge
- ✓ educational value

CONS

- ✓ low genetic variability (usually)
- ✓ selective genotypes upon cultivation
- ✓ hybridisation / genetic bottleneck
- ✓ relatively high intensity resources

Adapted from Yenson M AJ et al. 2021. Plant Germplasm Conservation in Australia: strategies and guidelines for developing, managing and utilising ex situ collections. Third edition. Australian Network for Plant Conservation, Canberra.

Questions to Ask To Determine the Most Efficient Way to Preserve the Plant Tissue Long-Term



Thanks!

ANY QUESTIONS?