联合国粮食及农业组织 | Food and Agriculture Organization of the United Nations | Organisation des Nations Unies pour l'alimentation et l'agriculture | Продовольственная и сельскохозяйственная организация Объединенных Наций | Organización de las Naciones Unidas para la Alimentación y la Agricultura | منظمة الأغذية والزراعة للأمم المتحدة

COMMISSION ON GENETIC RESOURCES FOR FOOD AND AGRICULTURE

Item 9.3 of the Provisional Agenda

Twentieth Regular Session

Rome, 24–28 March 2025

DRAFT PRACTICAL GUIDE FOR THE APPLICATION OF THE GENEBANK STANDARDS FOR PLANT GENETIC RESOURCES FOR FOOD AND AGRICULTURE: CONSERVATION THROUGH CRYOPRESERVATION

TABLE OF CONTENTS

1. Introduction

1.1 Introduction to cryopreservation

Plant genetic resources that are cryopreserved, stored at liquid nitrogen (LN) temperatures in a living state, have the potential to remain viable for decades at low cost and with minimal maintenance, without requiring regeneration. Cryopreservation technologies have been developed and implemented in numerous genebanks to preserve plant materials, particularly for security back-ups, that would be expensive to replicate in other forms, such as duplicates in field, greenhouse/screenhouse, or *in vitro* conditions (Panis *et al.*, 2020; Nagel *et al.*, 2024). Cryopreservation is a complicated, resource-intensive technology that has been successfully applied to secure materials in some genebanks. Successful cryopreservation programs require long-term funding and institutional support for infrastructure, skilled personnel, and equipment for both research and routine implementation. As a result, cryopreservation is not a required component for all genebanks.

Cryopreservation is not a required component for all genebanks

1.2 Collection types

Collections of plant genetic resources can be maintained under a range of conditions, with seed banks, field genebanks and *in vitro*/cryopreservation genebanks specifically listed in the *Genebank Standards for Plant Genetic Resources for Food and Agriculture* (Genebank Standards) (FAO, 2014). In the Genebank Standards, the seed bank standards cover the conservation of desiccation-tolerant orthodox seeds, where lower moisture and temperature decrease the rate of metabolic processes increasing seed longevity. Field genebank and *in vitro* conservation activities are aimed at the conservation of plants that are propagated vegetatively and may produce non-orthodox seeds, also known as recalcitrant or intermediate seeds. Field genebanks are also used for plants that produce very few seeds or that have a long life cycle to generate breeding and/or planting materials. It includes the maintenance of plants in the field, greenhouse/screenhouse, or shade houses (FAO 2014; Reed *et al.* 2004, Said Saad and Rao 2001). *In vitro* collections are usually materials that are propagated vegetatively and maintained using standard tissue culture or slow-growth (reduced temperature, low light, modified medium) conditions (Engelmann, 1999).

Seed bank, field genebank and *in vitro* collections (as defined above) are generally considered the active collections of the genebank. These are used for regeneration, multiplication, distribution, characterization, and evaluation. The base collection is a back-up of the active collection, which can be at -18ºC (for orthodox seeds) or cryostorage, depending on the type of material that is stored. It is recommended that base collections be safety duplicated at a secondary location (FAO, 2014) [1]. For orthodox seeds, this secondary location is often the Svalbard Global Seed Vault.

1.3 Genebank components

Some genebanks operate from a single facility/location for all activities, while others have a highly dispersed system with genebank units at multiple locations to place crop collections where they can be regenerated or maintained under appropriate field conditions.

Single location genebanks have the active and base collections co-located, facilitating the acquisition of materials for cryopreservation. However, this co-location could be a detriment if a natural disaster were to affect both the active and base collection at the same time.

In contrast, distributed genebank units within a larger genebank system have the advantage of locating crop collections in diverse conditions that are amenable to the crops, which is key for both seed regeneration and field maintenance of clonally propagated crops. Distributed genebank systems that

[1] Standards 4.9.1, 5.10.4, and 6.8.4.

employ cryopreservation technologies to secure collections may have a single location dedicated for cryopreservation activities, rather than duplicating the unique cryo-facility requirements at multiple dispersed locations. To ensure effective long-term storage, this arrangement requires careful coordination among multiple genebank units because materials must be shipped from the active site to the base collection for processing and storage.

1.4 Accessions and inventories

Within this guide, the term "accession" refers to a unique sample of plant genetic resources in the genebank, which could range from being a specific fruit tree cultivar in the field to a diverse bag of landrace seeds obtained from a market. The term "inventory" refers to one of multiple duplicates of that accession in the genebank. There could be a seed inventory in medium-term storage and another inventory for the same accession in long-term storage. Similarly, there may be multiple inventories (all genetically identical) of a fruit tree cultivar in the field, and additional inventories of the same cultivar stored in LN. In most cases, cryopreservation is used to make a longer-lived, safer inventory of an accession that is maintained elsewhere.

1.5 Base collections and the role of the cryobank

The establishment of secure base collections at a secondary location, either in cold storage, in the field, *in vitro*, or in a cryobank, is critical to reducing the risk of loss and makes it essential that accession and inventory information is carefully managed. Some genebanks rely on cryopreservation for preserving some of the base collection of an active collection. However, if materials are lost or removed from the active sites, the cryopreserved base collection materials may be the only remaining samples. Some genebanks may strategically use cryostorage to preserve their only inventories for some collections. This is particularly applicable for some recalcitrant and intermediate seed collections for which there may not be corresponding field inventories in the genebank.

1.6 Practical guides

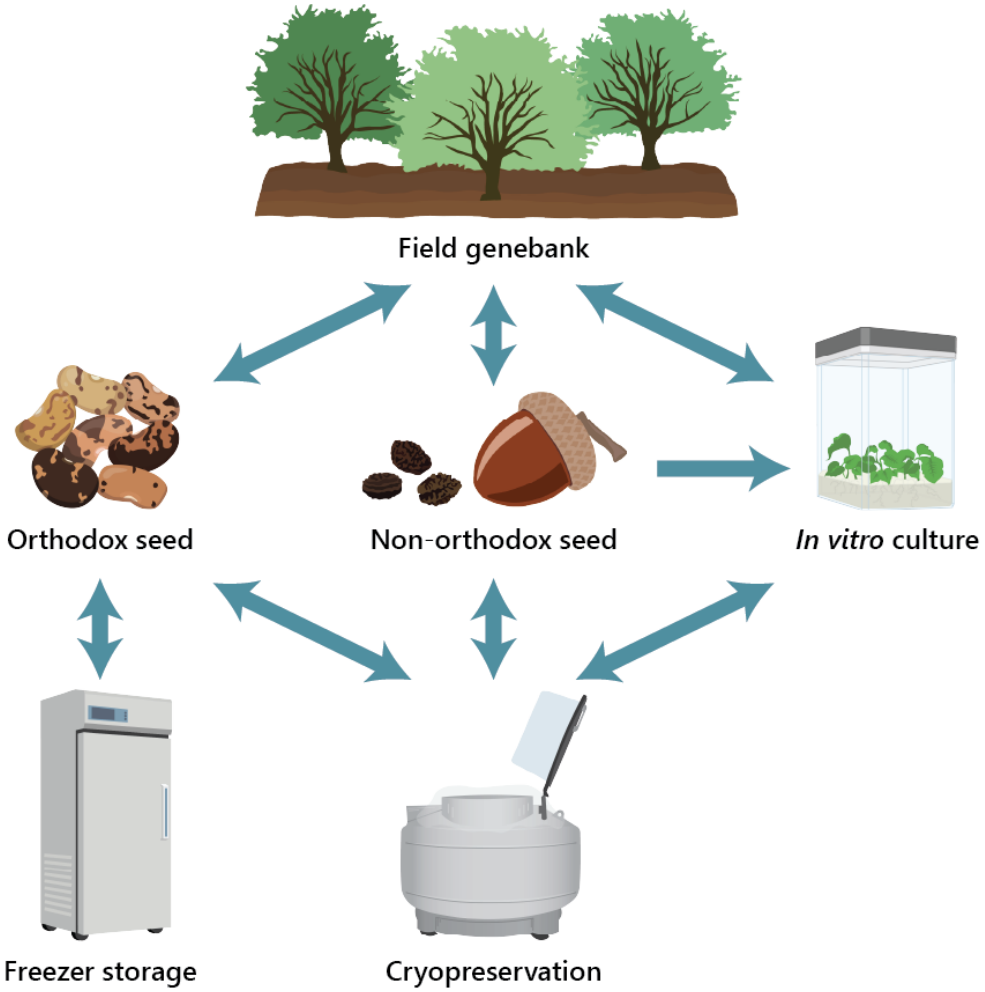
This *Practical guide for the application of the Genebank Standards for Plant Genetic Resources for Food and Agriculture: Conservation through cryopreservation* has been developed to be used as a companion volume to the *Genebank Standards for Plant Genetic Resources for Food and Agriculture* (FAO, 2014). This practical guide is directly linked to the other practical guide in this series.

Practical guides for the application of the *Genebank Standards for Plant Genetic Resources for Food and Agriculture* include:

1. Conservation of orthodox seeds in seed genebanks (FAO, 2022a)
2. Conservation in field genebanks (FAO, 2022b)
3. Conservation via *in vitro* culture (FAO, 2022c)
4. Conservation in genebanks of species producing non-orthodox seeds (FAO, 2025)
5. Conservation through cryopreservation

Cryopreserved collections may be composed of orthodox seeds, materials from a field genebank, materials from *in vitro* culture, or from species producing non-orthodox seeds. Cryopreserved collections may be comprised of materials, such as pollen, that are not included in the other practical guides. As a result, this practical guide for conservation through cryopreservation refers to the other practical guides, as appropriate (Figure 1).

Figure 1. Relationships among topics presented in the practical guides for orthodox seeds, field genebanks, *in vitro* culture, non-orthodox seeds, and cryopreservation.



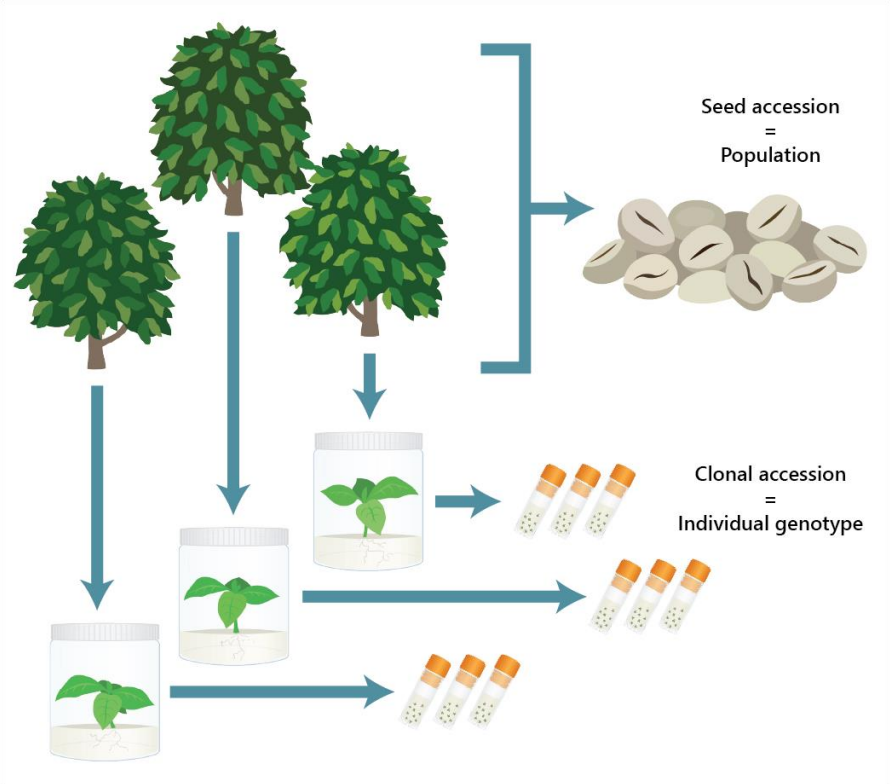
Source: Katheryn Chen, with permission

1.7 Conservation targets

The propagule choice to be cryopreserved depends on the plant species, the facilities available, the technical expertise, whether *in vitro*/cryopreservation protocols have already been developed, and on whether the genes or the specific genetic combination are the program conservation targets (Bettoni, Bonnart and Volk, 2021; Zhang *et al.*, 2023). One key consideration for plant genetic resource collections is whether the materials that are maintained (as growing plants, seeds, or in a cryopreserved state) capture the desired conservation target (Walters *et al.*, 2008). In some seed-propagated cultivars, seedlots are homogeneous, so that some to many of the individuals have the same genotype, according to the level of the self-fertilization (autogamy) of the species. In wild-collected seeds, such as for crop wild relatives, each seed often represents a unique genetic combination, thus there is a wide range of diversity in a single heterogeneous seedlot (Figure 2). Similarly, pollen grains are each unique genetic combinations of male gametes of an individual. In contrast, for clonally propagated crops, such as many fruit, nut, and some vegetable cultivars, all the individuals representing a cultivar are genetically identical because they are vegetatively propagated from the same original source plant (Figure 2). For these, each dormant bud or shoot tip collected from an individual (specific genotype) is genetically the same as all the others. Consideration of these factors is key when planning cryopreservation activities. Each of the cryopreserved explants of homogeneous cultivars are genetically identical, so a single individual successfully captures the conservation target. However, a larger quantity of seeds may be necessary to capture the diversity

represented in a highly heterogeneous seedlot during storage, regeneration, or for distributions. In some cases, seeds of these populations cannot be stored, so multiple genetic lines of clonally propagated cultures can capture the genetic diversity of a wild population (Figure 2).

Figure 2. Relationship among individuals that comprise a population and those that are preserved as individual genotypes=clones

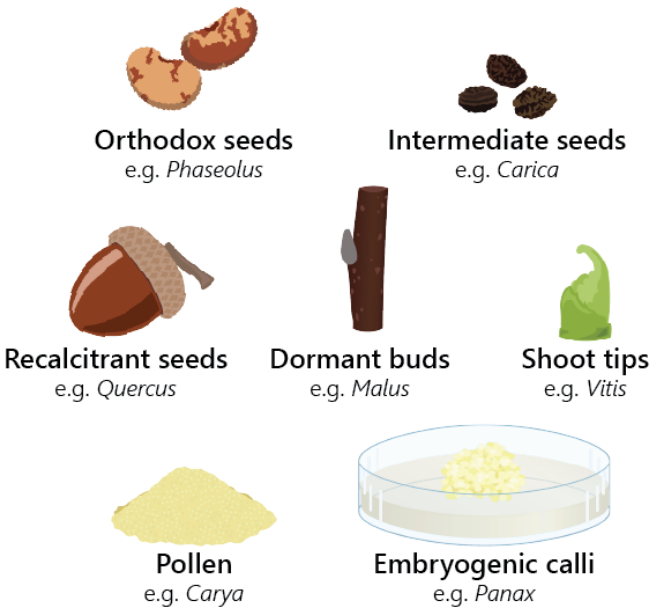


Source: Katheryn Chen, with permission

1.8 Propagules

Cryopreservation technologies can be applied to a wide range of propagule types, including orthodox seeds, intermediate seeds, and recalcitrant seed-derived embryos/embryonic axes, dormant buds, shoot tips, and pollen (Figure 3; Table 1). Many of these propagule types have the advantage of cryopreserving organs or regenerable groups of cells, which generally increases the likelihood of multiple cells contributing to the regeneration of a plant after cryo-exposure. Some propagule types, such as recalcitrant seeds (embryos/embryonic axes), have not been extensively implemented as cryopreserved propagules within genebanks, but there are active research programs with the intent of materials being cryobanked when methods/resources are available. In contrast, suspension cells and callus are undifferentiated cell types. Although they may be more easily cryopreserved than organized tissues, they also have a greater likelihood of somaclonal variation. Going through dedifferentiation to callus and then regenerating meristems can provide more opportunities for somaclonal variation than the direct outgrowth of cryopreserved meristems. Cryopreservation of suspension cells and callus (i.e. ginseng, Lei et al., 2021), as well as shoots derived from them and somatic embryos, are not covered in this practical guide. This guide includes the cryopreservation of desiccation tolerant pollen. Pollen that is desiccation sensitive can also be cryopreserved using specialized techniques (Nebot et al., 2021), but is not covered herein (i.e. Amphasys AG, 2022; Powerpollen, 2024).

Figure 3. Some types of plant propagules that can be cryopreserved



Source: Katheryn Chen, with permission

Table 1. Descriptions of some plant propagule types that can be cryopreserved.

Propagule type
Orthodox seed: Seeds that can be dried to low moisture content and stored at low temperatures to increase seed longevity. Homogeneous or heterogeneous seedlots are preserved.
Intermediate seed: Seeds that can be at least partially dried, but have shorter longevity at -18°C, and improved longevity when cryopreserved. Homogeneous or heterogeneous seedlots are preserved.
Recalcitrant seed: Seeds that are not desiccation-tolerant; they do not dry during the later stages of development and are shed at high water contents (in the range of 0.3 – 4.0 g g^{-1}). The loss of water rapidly results in decreased vigour and viability, and seed death occurs at relatively high-water contents. Homogeneous or heterogeneous seedlots are preserved.
Dormant buds: Plant buds in a physiologically arrested state during the winter months. Specific genotypes are preserved.
Shoot tips: Growing point of a plant that contains a meristem and surrounding immature leaves. Specific genotypes are preserved.
Pollen: Male gametes of plants that are either desiccation tolerant (able to survive in a dry state) or desiccation sensitive (unable to be dried below ~30% moisture content, fresh weight basis).
Callus: Undifferentiated plant cells usually derived from a single individual that can be treated to make somatic embryos or shoots. Specific genotypes are preserved.

1.9 Purpose of the practical guide

Conservation in genebanks by means of cryopreservation can be broken down into a series of interrelated operations (Figure 4). This practical guide presents practices and activities critical to each operational area and is based on the underlying principles of genebank management, namely: identification of accessions; maintenance of viability; maintenance of genetic integrity during storage and regeneration; maintenance of germplasm health; physical security of collections; availability, distribution and use of germplasm; availability of information; and proactive management (Table 2). It outlines workflows for routine genebank operations for conservation by cryopreservation and supports the application of the Genebank Standards. The purpose of this guide is to present the information contained in the Genebank Standards in a format that details the actions of the genebank workflow in a sequential manner and thereby facilitates more widespread adoption of the *Genebank Standards*. Genebanks may use the activities outlined in this guide as a basis for the development of standard operating procedures (SOPs) (Harding and Benson, 2015; IITA, 2012), best practices (Benson *et al.*, 2011a), and quality management systems (QMS) (Benson *et al.*, 2011b; CGIAR Genebank Platform, 2021) for conserving these germplasm collections, defining in detail how to carry out each activity. The publication "Refinement and standardization of storage procedures for clonal crops - Global Public Goods Phase 2: Part III. Multi-crop guidelines for developing *in vitro* conservation best practices for clonal crops" is a related guide that also provides valuable information relating to many of the same topics (Benson *et al.*, 2011b).

Figure 4. Major operations for conservation of germplasm through cryopreservation

Table 2. Underlying principles and related genebank operations for cryopreservation

Genebank principle	Summarized genebank operations
Identity of accessions	Passport data collected and recorded. Taxonomic and/or genetic identity verified. Permanent and unique accession number assigned and used in all documentation. Accessions handled carefully to avoid mixing, and all samples labelled and tracked throughout all genebank operations.
Maintenance of viability	Best practices followed and timing optimized during collecting, processing, introduction into cryopreservation, long-term storage, viability/regrowth assessment, and secure back-up storage. Cryopreservation conditions optimized and monitored. Germplasm health monitored regularly. Replacement undertaken when necessary.
Maintenance of genetic integrity	Collection and handling of samples conducted in a manner that ensures they represent the original population or clone. Best practices followed and genotypic data collected (when possible) to ensure sample identity and that no somaclonal variation or changes occurred during the cryopreservation, storage, and regrowth processes.
Maintenance of germplasm health	The stock-plants should preferably be free of fungi, bacteria, and viruses. Quarantine procedures undertaken when needed. Best practices followed during collection, processing, introduction into *in vitro* culture (if applicable), cryopreservation, and viability/regrowth. Contamination monitored and managed in the laboratory and in the field or greenhouse/screenhouse.
Physical security of collections	Risk management strategy developed and implemented. Appropriate genebank infrastructure in place and maintained. Accessions safety duplicated. Emergency or contingency plans in place.
Availability and use of germplasm	Germplasm acquired and distributed according to legal and phytosanitary requirements. Cryopreserved materials usually provided to genebank staff to replace accessions in the active collection or safety duplicates are returned to the original provider. Relevant documentation provided to recipients of genebank material.
Availability of information	Genebank information management system in place. Passport and accession management data secured by regular data back-ups. Passport and other relevant data available and accessible to external users, as appropriate.
Proactive management of genebanks	Standard operating procedures developed and available to staff. Data and information generated during genebank activities available to managers and staff. Well-trained staff employed and protected by occupational safety and health measures. Genebank staff capacities kept up to date and training provided as necessary. Cryopreservation and recovery methods are recorded and documented.

2. Considerations for cryopreservation

2.1 Choosing to cryopreserve

The specialized nature of cryobanks—facilities that preserve genetic resources in a cryopreserved state—along with the need for large, regular supplies of LN, can make establishing a cryobank prohibitively expensive for some genebanks. Building and equipping a cryopreservation facility requires reliable, guaranteed resources for technical support, maintenance, cryotanks, and LN. This long-term investment and dependence upon LN make some genebanks unable to develop cryobanks. Given the infrastructure required, genebanks should carefully consider their long-term capacity and whether it would be cost-effective to partner with regional or international centres for cryobanking needs. Regional cryobanks may serve designated geographic regions that include multiple national or international genebanks. These regional facilities may also be available as back-up locations for collections that are cryopreserved elsewhere. A global back-up cryopreservation facility has also been considered (Acker *et al.*, 2017).

Cryopreservation programs currently have research and implementation components. This is because the technology can sometimes be crop specific or difficult to implement directly from the literature. At a minimum, cryopreservation methods must be tested on a smaller scale before routine processing begins for large numbers of accessions. Genebanks must also consider the costs to acquire cryotanks, regular supplies of LN, employ skilled technical staff, implement an information management system, as well as a tissue culture facility (for materials that will be derived from or recovered as *in vitro* plants). This requires long-term institutional support.

Storing materials in cryotanks cooled by LN may not be the most cost-effective storage method for standard orthodox seeds that survive for decades or even centuries in freezer storage at -18°C.[2] As a result, genebanks that maintain only collections of orthodox seeds usually do not have associated cryopreservation programs. Genebanks with small field or *in vitro* collections may find it more cost-effective to duplicate field or *in vitro* collections at a secondary site rather than of introducing them into LN. In some cases, there may not be effective methods available to cryopreserve the crop of interest, thus a large investment is needed to first develop the method through research and then implement it.

2.2 Selection of crops & prioritizing materials for cryopreservation

If a genebank already has a cryopreservation facility, genebank staff must carefully consider many factors to identify and prioritize which crops and which propagule types will be cryopreserved (Table 3). Cryopreservation may be considered when duplicate collections are prohibitively expensive, methods are available and (ideally) routinely implemented in other genebanks, field collections are vulnerable due to abiotic or biotic stresses, seeds cannot be successfully stored in freezer conditions, *in vitro* collections do not retain their vigour, funding availability, uniqueness of the collection, or other factors that could prioritize a specific active collection for cryopreservation. In addition, access to adequate quantities and quality of propagules at appropriate physiological states should be considered. Consultations between active and base collection managers as well as genebank administrators and reviews of global crop conservation strategies may reveal collection vulnerabilities and cryopreservation priorities.

[2] Conserving orthodox or intermediate seed under cryopreservation is undertaken when seeds are not long-lived under conventional cold storage conditions.

Table 3. Considerations to identify crops or collections to cryopreserve

- ❑ Funding availability for cryopreservation
- ❑ Duplicate collections are prohibitively expensive
- ❑ Methods are available and (ideally) routinely implemented in other genebanks
- ❑ Access to adequate quantities and quality of propagules at appropriate physiological states
- ❑ Collection has been pathogen-tested and, preferably, free from pathogens (sanitized)
- ❑ Seeds cannot be successfully stored in freezer conditions
- ❑ Field collections are vulnerable due to abiotic or biotic stresses
- ❑ *In vitro collections do not retain their vigour*
- ❑ Collection vulnerabilities identified after reviews of global crop conservation strategies
- ❑ Uniqueness and difficulty to replace the collection
- ❑ Collection confirmed to be true-to-type
- ❑ Evaluation & characterization data are available

It is critical to select the appropriate propagule type to cryopreserve the desired conservation target. Propagules such as seeds or pollen may be more applicable to capture a larger portion of population genetic diversity than clones. Seed storage behaviour is often described as a continuum between extremely short-lived, desiccation-sensitive recalcitrant seeds and extremely long-lived orthodox seeds.

- Most orthodox seeds are stored at -18 to -20ºC for long-term storage. However, there are some shorter-lived orthodox seeds or samples that are very difficult to replace that are candidates for LN storage (Ballesteros *et al.*, 2021; FAO, 2025). For cryopreservation, orthodox seeds should be dried to about 32% relative humidity at 18ºC and then stored in the vapor phase of liquid nitrogen (LNV).

- Recalcitrant seeds do not tolerate removal of water that will freeze upon exposure to sub-zero temperatures, forming ice crystals that will damage the tissues. For recalcitrant seeds, cryopreservation of the whole seed is not usually possible when the seeds are large (>0.5 to 1 cm). For large seeds, the embryonic axes are excised and cryopreserved. The drying, cryoprotection, and cooling procedures must be adjusted, and are often species specific (Ballesteros *et al.*, 2021).

- Intermediate seeds have a storage response that is ‘intermediate’ to recalcitrant or orthodox seed storage behaviour. Intermediate seeds often survive drying at 50-65% relative humidity (RH) or lower but may be damaged if dried below 25% RH. They may lose viability faster in the freezer (-18°C) than in cold storage (4-8°C) (FAO, 2025). Examples include e.g., *Salix*, *Populus*, *Primula*, *Anemone*, and *Corylus* (Ballesteros *et al.*, 2021).

For clonal conservation of vegetatively propagated plants, dormant buds or shoot tips are preferred over seeds or pollen, as they can be established as whole plants with a high level of genetic fidelity and they also offer the opportunity to preserve selected individuals from the population that display desirable traits, such as disease resistance. Irrespective of the type of the propagule used, successful cryopreservation relies on high quality source plants, which therefore have a high regrowth capacity.

Some genebanks have collections that have been screened and sanitized to eradicate pathogens (fungi, bacteria, viruses, and/or viroids). In other cases, genebanks do not have the capacity to screen collections for pathogens, let alone clean them up. The cleanliness of collections should be considered as cryopreservation priorities are determined. Ideally, only sanitized and indexed collections are cryopreserved. The process of sanitization of some collections, may result in *in vitro* plants which could then be sources of materials for cryopreservation. If unclean collections are cryopreserved, then it may be necessary to re-process those materials for cryopreservation if they are sanitized at a later date.

In addition, some crop collections in genebanks have been genotyped and/or phenotyped to determine if they are true-to-type with respect to taxonomy and cultivar. These collections that are confirmed to be true-to-type could be considered as priorities for cryopreservation.

2.3 Method development

Cryopreservation method development can range from straightforward to a long-term endeavour. For orthodox (including short-lived orthodox, which might be referred to as intermediate) seeds, methods are generally available and readily applicable. For recalcitrant seeds, clonal propagules, and pollen, methods must be identified through research. This research must identify optimum media for plant *in vitro* introduction, multiplication, and regeneration, as applicable, depending upon the propagule type. In addition, methods and the extent of desiccation, cryoprotectant pre-treatment, cryoprotectant solution treatment, LN exposure, packaging, and regrowth must all be optimized. This process of optimization requires many experiments with appropriate controls to determine the necessary parameters.

Cryobiology is a state-of-art scientific discipline that is constantly evolving. New and improved methods are continuously being developed to preserve an ever-broader range of species and propagule types. One challenge is that methods must often be optimized for the particular species, or even genotype within the same species, and/or crop, and source plant conditions. As a result, methods must be tested in-house to confirm that they can be successfully applied prior to large-scale implementation. Applied research and testing may take months or years. If methods have not been previously developed, it often requires years of experimentation to develop new methods—and genetic resources of some crops have never been successfully cryopreserved despite decades of effort.

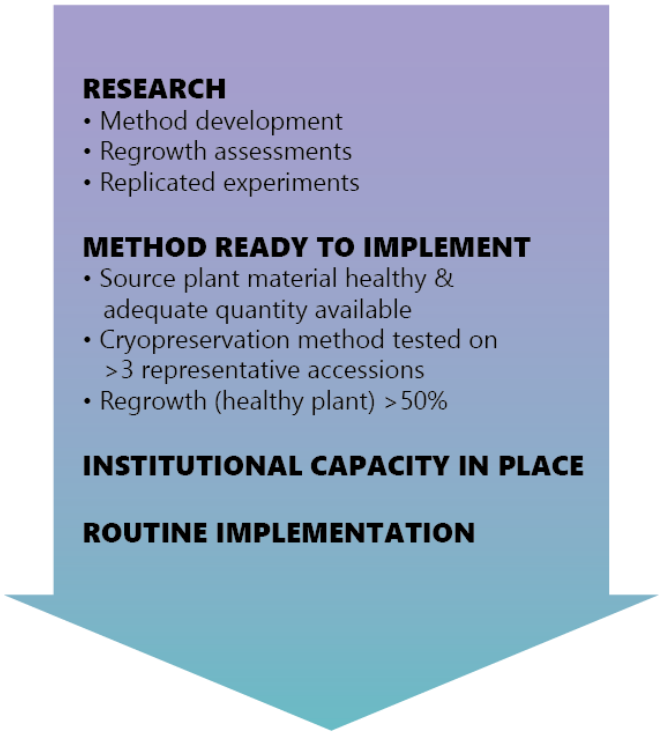
Numerous reviews and best practices (such as Reed, 2008) have provided information about the key features of plant cryobank facilities, as well as how to select and optimize methods for successful cryopreservation, and these are noted in the text. Some examples are provided from preservation facilities including Kew Royal Botanical Gardens Kew (UK), the USDA-ARS National Laboratory for Genetic Resources Preservation (NLGRP; Fort Collins, Colorado, USA) and the International Potato Center (CIP; Lima, Peru; Vollmer *et al.*, 2016).

2.4 From research to implementation

Generally, methods are developed for 4-5 accessions that are representative of the desired range of genetic diversity within a crop/species. The process of determining if a method is ready for implementation is called ‘validation’ (Benson *et al.*, 2011b). If plants can be successfully regenerated with viability levels >50% (ideally higher) using the identified method, then the method may be ready for larger-scale implementation (Figure 5). In some cases, specific cultivars may not be amenable to the identified procedures and modifications may be needed. Some examples of crops for which cryopreservation has been widely implemented include coffee (intermediate seeds; Dussert *et al.*, 1998), apple dormant buds (Höfer, 1998), and banana, potato, mint, and garlic shoot tips (Nagel *et al.*, 2024).

Although it may be labour-intensive and expensive to introduce materials into cryostorage, once cryopreserved, the annual cost of their preservation in LN is often much less than the cost of maintaining duplicate field, greenhouse/screenhouse, and *in vitro* plant collections, or regenerating seeds frequently due to their short storage life and is, above all, the safest strategy for the long-term storage of plant genetic resources, complementing traditional field genebanks and *in vitro* collections. In some cases, genebanks may retain only the cryopreserved accession when the field or *in vitro* materials are no longer living or when the field or *in vitro* collections are purposefully downsized.

Figure 5. Depiction of the process from research to routine implementation of cryopreservation



Source: Katheryn Chen, with permission

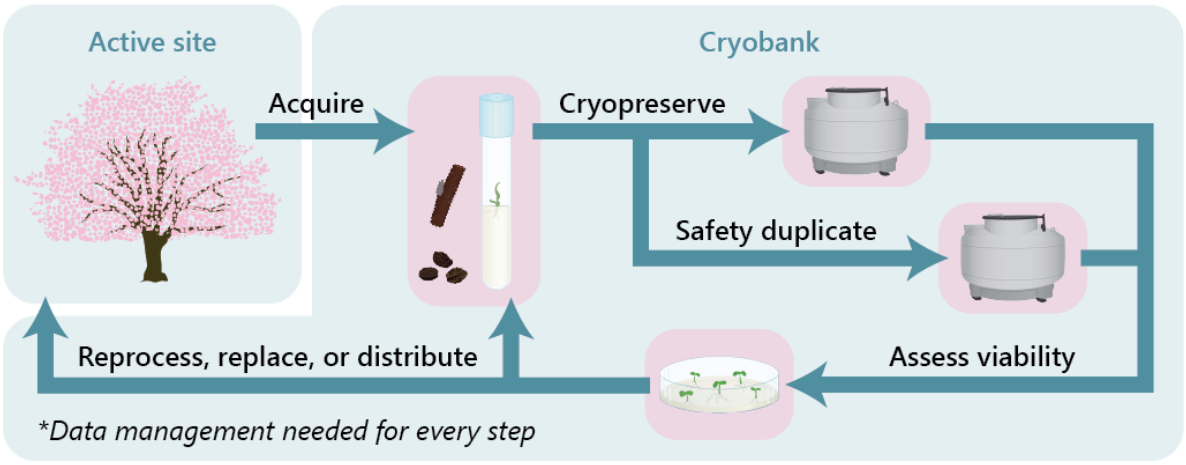
2.5 When is a collection safely cryopreserved?

Cryopreserved collections are usually base collections (back-ups) of active collections. Genebanks must determine their priority materials to be cryopreserved and the criteria, or standards, to determine if the material is secure.

2.6 Relationships among conservation activities relating to cryopreservation

Activities relating to cryopreservation are not the same as those necessary for orthodox seed, field, and *in vitro* collections. This guide includes sections for many topics that relate cryobank activities (Figure 6), including: 1) Acquisition of materials for cryopreservation or that are already in the cryopreserved state; 2) Cryopreservation of materials when a method is already available; 3) Long-term storage of propagules in either liquid nitrogen (LN) or the vapor phase of liquid nitrogen (LNV); 4) Viability/regrowth assessment, periodic monitoring of viability/regrowth, and post-cryopreservation quality assessment; 5) Replacement of cryopreserved inventories (when necessary); 6) Distribution (usually to replenish materials within the genebank); 7) Safety duplication of cryopreserved materials; and 8) Documentation.

Figure 6. Relationships among activities covered in the cryopreservation practical guide



Source: Katheryn Chen, with permission

This booklet only provides general guidance on the complex steps and decisions required when operating a genebank for cryopreservation. Each genebank will have its own circumstances, and the efficient management of particular collections will require careful consideration and procedural adjustments based on experience. For detailed technical specifications of the steps outlined in this guide, genebank staff will need to consult various sources of information, a few of which are referenced in this booklet.

3. Acquisition of germplasm

The genebank is recommended to have documented policies and/or procedures, as applicable, for acquiring germplasm that include abiding by legal, phytosanitary and other regulations and requirements.[3]

Materials to be cryopreserved are usually sampled from a genebank's existing collections, where seeds and other propagules received through explorations and donations were originally received by the curators of the main collections.[4] In some cases, materials may be received by other cryo-facilities in the cryopreserved state. In these cases, propagules must remain cryopreserved throughout the entire transfer process. Detailed information about the acquisition of orthodox seeds, non-orthodox seeds, and clonally propagated materials is available in other FAO practical guides (FAO 2022a, 2022b, 2022c).

✓ **Decisions to accept germplasm into a genebank's collection are guided by the institute's acquisition policy.**

The development of an acquisition policy ensures that collections remain manageable and meet users' needs (Guarino, Rao and Reid, eds., 1995).

- Genebank curators may interact with breeders, botanists and other scientists before deciding on new acquisitions. Institutes may also have a crop-specific or general advisory committee in place.
- The health and viability status of collected or donated samples, availability of passport information (taxonomic identity, origin of the germplasm, etc.) and sample "uniqueness" (to avoid unnecessary duplicates) should also be considered in the decision-making process and are determinants for increasing the efficiency of collection preservation efforts.

✓ **Germplasm added to the collection is legally acquired and accompanied by all relevant documentation.[5]**

The process of germplasm acquisition is governed by national and international regulations such as phytosanitary/quarantine regulations and the International Treaty on Plant Genetic Resources for Food and Agriculture (Treaty) or the Convention on Biological Diversity (CBD) for access to genetic resources (FAO, 2014).

- The genebank should communicate with the National Focal Points for the Treaty or other designated authorities on questions concerning germplasm acquisition.

✓ **A permanent and unique accession number is assigned to each sample added to the genebank collection.**

Once the curator decides to accept a sample into the genebank, a unique accession number must be assigned.

- A Digital Object Identifier (DOI) can be requested from the Secretariat of the Treaty (FAO, 2021a). Both the accession number and the DOI remain with all material derived from the accession during all genebank handling.
- If the donated material has an accession number assigned by the donor organization, a DOI, or both, keep these as alternative identifiers in the passport data. This is a critical means of ensuring the unambiguous association of information with the material.

[3] See Table 4 at the end of this section for a summary of the workflow and activities for acquisition of germplasm.
[4] Specific guidance is available for acquisition of orthodox seeds (FAO, 2022a), cuttings and vegetative materials (FAO, 2022b), materials maintained *in vitro* (FAO, 2022c), and recalcitrant and intermediate seeds (FAO, 2025).
[5] Standard 6.1.1.

✓ **Germplasm added to the genebank collection is accompanied by associated data, as outlined in the FAO/Bioversity Multi-Crop Passport Descriptors.[6]**

It is recommended that all new accessions, whether obtained through collection missions or donation from other institutes, be accompanied by the associated data detailed in the FAO/Bioversity Multi-Crop Passport Descriptors (Alercia, Diulgheroff and Mackay, 2015).

- The association of data and associated inventories with the single accession must be clear, for example by using accession numbers and/or DOI.

✓ **All acquisition data, including associated metadata, are recorded, validated and uploaded to the genebank's information management system.**

Consider the use of electronic devices to avoid transcription errors and for ease of uploading. Otherwise, the use of indelible ink (or pencil) and clear, legible writing are necessary when recording data. The use of barcode labels and barcode readers facilitates accession management, speeds up the process and minimizes human error.

3.1 Transfer of materials within a genebank

Plant materials may need to be transferred between the active site and the cryobank within a genebank system or between one genebank and a regional cryobank. When materials must be transported, specific protocols must be followed.

✓ **Protocols for collecting, packaging and shipping seeds and other propagules are followed depending on the target species and through conversations with the cryopreservation program.**

- Protocols are available for collecting orthodox seeds (FAO, 2022a) and for collecting propagules from species producing non-orthodox seeds or that are vegetatively propagated (FAO, 2022b; FAO, 2022c; FAO, 2025).
- Pollen is collected from either populations of plants or from single individuals, depending on the quantity and availability of inflorescences. Pollen preservation is most commonly performed for species with desiccation-tolerant pollen (Ballesteros and Pritchard, 2020). The process of collection is dependent upon species. In some cases, just the male (pollen containing) inflorescence will be collected. In others, entire flowers will be collected. The reproductive stage of flower maturation for successful pollen collection is dependent upon species. Collected materials must be air dried and sifted as soon as possible after collection to release the pollen (Rajasekharan and Rohini, 2023).
- To prevent loss and deterioration of the material, the time between collection, processing and transfer to the genebank should be as short as possible (FAO, 2022b; section 3).

✓ **Materials should be shipped overnight if genebank is in a different location, or as quickly as possible and packaged to ensure safe delivery.[7]**

- Use rigid, insulated packing material.
- Sending shipments using the fastest means possible, by airfreight or courier, should be used to avoid exposure to adverse environmental conditions and deterioration of quality.
- A high degree of coordination between the sender, the shipping company and the receiving genebank is required. The shipping number and tracking information should be shared with the genebank staff; genebank staff should regularly check where packages are and when they are expected to arrive. Genebank staff should be ready to process the samples immediately upon arrival at the genebank.

✓ **Visual inspection of the surface of fruits/seeds/buds and under the covering tissues is made, to detect signs of pathogens and insects.**

[6] Standard 6.2.2.
[7] Standard 6.2.4.

- Look for obvious signs of fungi such as discolouration and 'furry' fungal colonies.
- Check for holes in the seed coat / covering structures which may indicate the presence of insect larvae and/or eggs, and damage of internal tissues.
- Discard infected / infested seeds / buds according to normal biological waste management procedures; it may be necessary to destroy entire seed lots, if contamination rates are high.

✓ **Material arriving at the cryobank is checked for damage/contamination and that it is accompanied by the appropriate documentation.**

All material arriving at the genebank is visually checked for damage/contamination by an authorized and knowledgeable person in a designated reception area and immediately placed into appropriate environmental conditions. Cross-checking should also be made to ensure the material is as described in the accompanying documentation (which may be sent in advance electronically).

- Visual inspection of the surface of fruits/seeds and under the covering tissues is made, to detect signs of pathogens and insects.
  - Look for obvious signs of fungi such as discolouration and 'furry' fungal colonies.
  - Check for holes in the seed coat / covering structures which may indicate the presence of insect larvae and/or eggs, and damage of internal tissues.
  - Discard infected / infested seeds according to normal biological waste management procedures; it may be necessary to destroy entire seed lots, if contamination rates are high.
- Decontamination activities, such as treating samples with a surface disinfectant agent, are used (as needed and appropriate) to remove all adherent microorganisms, taking into account any decontamination treatment given prior to packaging and transport.[8]
- Quarantine measures are applied as necessary.
- Inventories are confirmed to ensure that all the expected materials arrived.
- All incoming materials should be handled as soon as it arrives at the genebank and temporarily stored under conditions appropriate for that propagule type.
  - Orthodox seed should be cleaned and dried for temporary storage (see FAO, 2022a; section 3).
  - Recalcitrant seeds should be removed from structures as necessary, cleaned and maintained under short-term humid storage (see FAO, 2025; section 3). For recalcitrant species, intact fruits and seeds should be aerated and perhaps transferred to new, clean loosely-tied polyethylene bags, or sterile plastic buckets/boxes, while still maintaining the moisture content as far as possible: at 6±2°C unless the seeds/fruits are of a species known to be susceptible to chilling injury, for example, *Theobroma cacao* which should be kept at 17-30°C (Hor, Chin and Karim, 1984).
  - Roots, tubers, cuttings, bulbs, and dormant buds should be kept in storage under cool temperatures in a designated storage area free of pests (see FAO, 2022b; section 6). These materials are then used as source materials for explant excision or introduced *in vitro* for multiplication and cryopreservation.
  - *In vitro* materials should be stored under optimum storage conditions (See FAO, 2022c).
    - Depending on the condition of the sample, it may be necessary to undergo multiplication prior to storage.
  - Flowers/pollen are received in a dry state or harvested locally and dried on site. Inflorescences are sifted to remove flower parts and any insects. Moisture content

[8] Standard 6.1.5.

is then adjusted and pollen is prepared for immediate cryopreservation (see section 4).

3.2 Materials transferred to cryobank from another genebank for safety duplication

The Genebank Standards recommend that cryopreserved materials are safety duplicated at a secondary location in the cryopreserved state (FAO, 2014; see Chapter 8). As such, genebanks with cryostorage facilities may receive germplasm from another cryobank for the purpose of safety duplication. It is therefore important that the receiving genebank has the long-term capacity to maintain cryopreserved collections.

✓ **All necessary agreements and documentation are in place prior to receiving materials.**

- Agreements should consider duration, best practices, and costs.
- Ensure that the genebank receives the required documentation (both for the genebank and the host country), and the applicable customs and phytosanitary procedures. This will help ensure timely movement of the germplasm.
- The genebank is encouraged to use a Standard Material Transfer Agreement (SMTA) (FAO, 2021b, c).

✓ **Material sent to the cryobank is prepared, packaged and sent in a way that ensures material arrives intact and viable.**[9]

- Request that shipments are sent using the fastest means possible, by airfreight or courier, to avoid exposure to adverse environmental conditions and deterioration of quality.
- Continuous tracking of the package, if possible, will ensure that cryobank staff are prepared to process the samples upon arrival at the cryobank.

✓ **Material arriving at the cryobank is kept at LN or LNV temperatures throughout the transfer process.**

- Phytosanitary and quarantine regulations are followed, without warming the cryopreserved materials.

✓ **Received materials are documented.**

- Inventories are confirmed to ensure that all the expected materials arrived.
- Provider receives confirmation that materials arrived successfully and their storage location.

[9] Standard 6.1.4.

Table 4. Summary of the workflow and activities for the acquisition of germplasm

Acquisition of germplasm	
Germplasm added to the collection is legally acquired and abides by national, regional and international phytosanitary and any other import regulations and requirements.	- Follow legal requirements: national regulations, International Treaty on Plant Genetic Resources (Standard Material Transfer Agreement); Convention on Biological Diversity (prior informed consent and mutually agreed terms). - Follow phytosanitary requirements: import permit; phytosanitary certificate.
Germplasm is obtained from a genebank's own collection maintained at a different location from cryopreservation facilities.	- Collect germplasm maintained in the field based on breeding system. - Collect from visibly healthy plants. - Ensure correct physiological age/stage for successful processing. - Carefully label and avoiding mixing samples. - Ensure short interval between collecting and transfer to genebank.
Germplasm is transported between the active site and the cryobank.	- Use rigid, insulated packing material. - Provide packing lists and coordinate shipments between sender and recipient. - Use overnight shipment when possible.
Safety duplicates are from another cryobank are received.	- Have all necessary agreements in place for legal transfer of materials. - Ensure that cryopreserved materials remain at LN temperatures throughout the shipping process. - Inventory and document the materials received. - Provide storage location information to the provider.
Record, validate and upload all cryopreservation data, including associated metadata.	

4. Cryopreservation of diverse propagule types

The genebank is recommended to have documented policies and/or procedures, as applicable, for the conservation of germplasm through cryopreservation, including guidelines for the preparation of materials for cryostorage.[10]

Cryopreservation procedures and considerations are dependent upon the species and propagule types to be conserved. Generally, methods of protecting cells focus on generic protocols for seeds and species-specific protocols for clonal propagules. As such, there are no one-size-fits all protocols and individual protocols go beyond the scope of this guide. It may be necessary to carry out a literature review to investigate whether protocols exist for each step of the cryopreservation process based on the species and type of propagule used and the facility available. If information is not available for a given species, it may be useful to test protocols for a related species. Methods derived from the literature should be thoroughly tested across a wide range of genetic diversity and optimized prior to implementation[11] (see Benson *et al.*, 2011a, b; Vollmer *et al.*, 2019; Ballesteros *et al.* 2021; Bettoni, Bonnart and Volk, 2021; Nagel *et al.*, 2024;). The below provide some basic guidance of tested procedures.

- ✓ **The methodology to prepare samples for cryostorage is determined based on the species, propagule type, and conditions of the source material.**

- ✓ **Only viable and disease-free materials are introduced into cryopreservation**.[12]

- ✓ **Clean and sterile containers for cryostorage are used.**

- ✓ ***In vitro* source plants that have not been subcultured for extended lengths of time are used as donors of propagules for cryopreservation, if possible.**

- ✓ **Adequate quantities of propagules are cryopreserved to ensure that genetic shifts do not occur if there is a decline in viability during storage.[13]**
    - Fewer propagules may be cryopreserved for clonally propagated materials, such as dormant buds and shoot tips, because each of the propagules in the cryopreserved inventories are genetically identical, and as a result, only one successful regrowth event is needed to regenerate an individual identical to the original source plant.

- ✓ **Samples are protected from lethal ice formation by air drying or application of cryoprotectants.**

- ✓ **Samples are packaged into vessels and materials suitable for storage in LN, such as cryovials, based on the propagule type, size and protocol used.**

- ✓ **Secure labels that will remain adhered to the package at LN temperatures are affixed to the packages.**

- ✓ **Samples are cooled fast enough to avoid intracellular ice formation during the cooling or warming process.**

- ✓ **A temporary holding cryotank is used to store cryopreserved samples prior to viability testing, and if viability reaches the cryobank parameters, the cryopreserved samples are transferred to the permanent cryotanks.**

[10] See Table 5 at the end of this section for a summary of the workflow and activities for cryopreservation of diverse propagule types.
[11] Standard 6.5.2.
[12] Standard 6.5.1.
[13] See section 6.7

✓ **Viability/regrowth assessments are performed on materials immediately after LN exposure to ensure that the cryopreservation procedure was successful[14]**

✓ **Samples are placed into LN/ LNV for long-term storage.**

When placing into LN/LNV, it is important to follow the following steps:

- Unlock the cryotank and very gently lift the lid. If the lid is opened suddenly excess fogging will occur making it difficult to locate the appropriate rack.
- Transfer materials to the cryotank quickly to ensure no warming occurs.

✓ **All cryopreservation data, including associated metadata, are recorded, validated and uploaded to the genebank information management.**

Data to consider are provided below.

- Inventory identifiers that are associated with source accession
- Propagule type
- Propagule harvest date, and other information related to sample collection (storage time, shipping records, etc.)
- Cryopreservation date
- Storage vessel (cryovial, etc.)
- Number of propagules per storage vessel
- Number of storage vessels in LN
- Location of storage vessels in cryotank
- Detailed methods for cryopreservation and viability/regrowth
- Viability/regrowth level of inventory
- Total number of viable propagules in cryostorage for each inventory
- Cryotechnician who processed the material

4.1 Cryopreservation of orthodox seeds

Some orthodox seeds lose viability too quickly in conventional seed genebank storage for freezer storage to be an effective method for long-term ex situ conservation. Cryopreservation of such seeds is recommended. There may be other reasons for conserving orthodox seeds at very low temperatures, for example, if the seed sample represents a particularly valuable species from which it is difficult to collect seeds. Procedures for LN storage of orthodox seeds differ from standard -18 °C storage procedures. In particular, to avoid a potential risk of over drying when seeds are dried at ambient temperature and then stored at LN temperatures, seeds are not dried to such low moisture contents as used for conventional seedbank storage (Ballesteros *et al.*, 2021). Optimum conditions (moisture content and cooling rate) that provide high levels of initial survival and seed stability during long-term storage are currently under investigation (C. Walters, personal communication).

✓ **Seeds are subjected to initial viability testing following optimized and documented procedures.**

- Seeds to be cryopreserved should be of high quality and high viability.
- It is important to use standard protocols so that viability monitoring tests are comparable, including over time, ideally using replicated testing procedures (section 6; see FAO, 2022a).

✓ **Seed samples are equilibrium dried to optimum moisture content for cryostorage.**

- In general, orthodox seeds dried to 60% RH and below survive short term exposure to LN. The optimal moisture content for storage varies among species, and even within species,

[14] See section 6.

(Nits *et al.*, 2024) but generally, adjustment to 30% RH should ensure seeds of most species are dried to acceptable moisture content (FAO, 2025; C. Walters, personal communication).
- Moisture content can be manipulated by placing seeds in a room with controlled relative humidity and temperature or by placing seeds in chambers with saturated salt solutions. The humidity level within the chamber is dependent upon the salt selected (Pritchard and Nadarajan, 2008).

✓ **Seeds are assessed for moisture content to ensure they are adequately dry.**
- Moisture content of the sample is determined if sufficient quantity of material is available (see FAO, 2025).
- The recommended sample size per replicate is 0.5-1.0 g for small seeds and 4.5-5.0 g for larger seeds (cereals, legumes). Moisture content determination should be performed with minimum 2 independent replicates, and 3-4 replicates for greater accuracy.

✓ **Seeds are packaged into materials suitable for storage in LN, such as cryovials, based on size.**
- If enough seed and resources are available, it is recommended that seeds be aliquoted into multiple packages to simplify the distribution and/or viability assessment processes.
- The quantity of seeds stored is dependent upon seed size, quantity available, storage space available, seedlot heterogeneity, the number of seeds utilized for viability assessments, and the anticipated number of seeds needed for distributions to the active site.

✓ **Secure labels that will remain adhered to the package at LN temperatures are affixed to the packages.**
- Bar codes or labels with indelible ink, suitable for use in LN, with inventory information may be applied for ease in sample identification.

✓ **Seeds are cooled to storage temperature.**[15]
- In some cases, a slower cooling rate may be beneficial to prevent damage during introduction into LN.
- Slower cooling (<10C/min) tends not to lead to mechanical damage.

✓ **Seeds are stored in LNV (in most cryobanks).**

4.2 Cryopreservation of intermediate seeds

Intermediate seeds can tolerate drying, but due to their shorter longevity, cryopreservation may be a preferable storage temperature. Intermediate seeds may be cryopreserved as either whole seeds or as excised embryos, most often depending upon seed size. Because the seeds are able to tolerate drying, general procedures may be similar to those of orthodox seeds. A key feature of intermediate seeds is their desiccation tolerance to well below where the cytoplasm naturally vitrifies (C. Walters, personal communication).

✓ **Seeds are subjected to initial viability testing following optimized and documented procedures.**
- Seeds to be cryopreserved should be of high quality and high viability.
- It is important to use standard protocols so that viability monitoring tests are comparable, including over time, ideally using replicated testing procedures (see FAO, 2022a; section 4).

Preparation of intermediate seeds that are cryopreserved whole

✓ **Seed samples are equilibrium dried to optimum moisture content for cryostorage.**

[15] See section 5 on long-term storage in liquid nitrogen.

In general, intermediate seeds dried to 50% RH and below survive short-term exposure to LN. The drying levels for LN storage of intermediate seeds are higher than those used for standard storage at -20 ºC to avoid drying damage (C. Walters, personal communication). NLGRP adjusts seed moisture levels for cryopreservation by placing seeds at known RHs (50% for intermediate seeds).

✓ **Seeds are assessed for moisture content to ensure they are adequately dry.**

- Moisture content of the sample is determined if sufficient quantity of material is available (see FAO, 2025).
- The recommended sample size per replicate is 0.5-1.0 g for small seeds and 4.5-5.0 g for larger seeds (cereals, legumes). Moisture content determination should be performed with minimum 2 independent replicates, and 3-4 replicates for greater accuracy.

Preparation of intermediate seeds that are cryopreserved as excised embryos/embryonic axes

Intermediate seeds that are large (>0.5 to 1 cm) cannot be cryopreserved intact, primarily because the internal tissues would not cool fast enough.

✓ **Embryos/embryonic axes are excised from seeds as soon as possible after the seeds arrive at the genebank.**

Only healthy-looking fruits/seeds with no signs of disease or damage should be used.

- Often seed material is disinfected and the axis isolated in a laminar flow hood. Thereafter, all steps in the cryopreservation protocol are sterile. Alternatively, disinfection is applied after the cryopreservation process on entry to tissue culture for regrowth.
- Excision of the explant should be optimized, as cutting too close to the apex of an embryonic axis can result in damage and poor subsequent growth. Explant size is dependent upon species and protocol.
- Excised embryos / embryonic axes should be immediately placed in antioxidant solution.
    - An example of antioxidant solution is 50 mg / L ascorbic acid in water or liquid culture medium.

✓ **The number of embryos/embryonic axes excised and stored is determined by the number of seeds available, heterogeneity of the seedlot, and the number needed to be placed in long-term storage and for viability assessments.**

✓ **Propagule water content is reduced through controlled air drying, encapsulation-dehydration or vitrification or encapsulation-vitrification**

- Air drying of recalcitrant seed embryonic axes is carried out with freshly regenerated silica gel desiccant or in the flow of sterile air in a laminar hood before deep cooling (see Ballesteros *et al.*, 2021).
- Encapsulation-dehydration, vitrification, or encapsulation-vitrification are species-specific procedures developed for cryopreserving recalcitrant seed embryos or embryonic axes (see Ballesteros *et al.*, 2021).

✓ **For either seeds or embryos/embryonic axes, materials are packaged into containers suitable for storage in LN, such as cryovials, based on the propagule type.**

- If enough seed and resources are available, it is recommended that seeds be aliquoted into multiple packages/cryovials to simplify the distribution and/or viability assessment processes.
- Packaging should consider the need to retrieve samples without warming the remaining samples.

✓ **Secure labels that will remain adhered to the package at LN temperatures are affixed to the packages.**

- Bar codes or labels with indelible ink, suitable for use in LN, with inventory information may be applied for ease in sample identification.

✓ **Seeds are cooled to storage temperature.**[16]

- In some cases, a slower cooling rate may be beneficial to prevent damage during introduction into LN. This may help avoid cracking during the cooling process.
- Slower cooling (<10C/min) tends not to lead to mechanical damage.

✓ **Seeds are stored in LNV (in most cryobanks).**

4.3 Cryopreservation of recalcitrant and large intermediate seeds

Recalcitrant seeds cannot usually withstand sufficient drying for storage at LN temperatures. Recalcitrant seed cryopreservation depends on finding a balance between avoiding desiccation damage and having cooling rates fast enough to prevent ice formation. This usually requires the excision of embryos/embryonic axes. Some information relating to the cryopreservation of recalcitrant seeds for specific taxa may be available in the literature. Taxa-specific methods may take into account the size of the tissue, water relations of embryonic axes, and stress-related metabolism (Walters *et al.*, 2008).

✓ **Embryos/embryonic axes are excised from seeds as soon as possible after the seeds arrive at the genebank.**

✓ **Source materials from healthy, mature seeds are used, selecting axes free from mechanical damage and considering the developmental stage of the embryo.**

✓ **The number of embryos/embryonic axes excised is determined by the number of seeds available, heterogeneity of the seedlot, and the number needed to be placed in long-term storage and for viability assessments and is an individual genebank decision.**

✓ **Healthy-looking fruits/seeds with no signs of disease or damage are used.**

- In some cases, the fruits/seeds are surface sterilized and excised under sterile conditions. If materials are surface sterilized, it may include treatment with diluted alcohol (ethanol or isopropanol) and/or sodium hypochlorite solutions, followed by sterile water.
- Excision of the explant should be optimized, as cutting too close to the apex of an embryonic axis can result in damage and poor subsequent growth. Explant size is dependent upon species and protocol.
- Large embryos/embryonic axes, such as those for *Persea*, may require the use of the plumule.
- If materials are not excised under sterile conditions, disinfection is applied after the cryopreservation process on entry to tissue culture for regrowth.
- Generally, successful cryopreservation is achievable in small embryonic axes (< 2 mg) that have a water content (WC) <= 1.5 g H_2O/g dry weight, equivalent to water potential (WP), WP = -0.8 MPa and that don't turn brown too much upon excision (C. Walters, personal communication).

✓ **Propagule water content is reduced through controlled air drying, silica gel, encapsulation-dehydration or vitrification or encapsulation-vitrification**

- Drying of recalcitrant seed embryonic axes is carried out with freshly regenerated silica gel desiccant, in the flow of sterile air in a laminar hood or air-drying in a dry climate before deep cooling (see Ballesteros, 2021).

[16] See section 5 on long-term storage in liquid nitrogen.

- Encapsulation-dehydration, vitrification, or encapsulation-vitrification are species-specific procedures that have been reported in the literature for cryopreserving seed embryos or embryonic axes of some recalcitrant seed species (see Ballesteros, Fanega-Sleziak and Davies, 2021).

✓ **Seeds are cooled to storage temperature.**[17]

- Fast cooling rates, either with or without cryoprotectant treatment, may achieve successful cryopreservation without ice crystal formation (Walters *et al.*, 2008). One example of seed cooling procedures is provided by Walters *et al.* (2008).

✓ **Seeds are stored in LNV (in most cryobanks).**

4.4 Cryopreservation of dormant buds

Dormant buds of temperate-adapted woody plants may be cryopreserved to provide a back-up of specific genotypes (i.e. cultivars) in field collections. Although restricted to a limited number of species, dormant bud cryopreservation is generally much less labour intensive than shoot tip cryopreservation. However, using dormant buds introduces the possibility of cryopreserving virus (if the mother plant was infected), which will require an additional virus eradication procedure to obtain clean plants. Dormant buds are harvested when the plants are fully winter-dormant, prior to the time that dormancy is released. Some growth locations may not result in the dormant buds achieving adequate dormancy for successful cryopreservation of dormant buds. Ideally, twigs containing dormant buds from the previous growing season are harvested after several days of low temperatures. For detailed information, see Volk, Jenderek and Chen (2020b) and Tanner *et al.* (2021a).

✓ **Dormant twigs containing dormant buds are harvested, bundled, placed in sealed plastic bags, and stored in the 0-5ºC cooler until shipment or use.**

It is important that twigs are processed as soon as possible after harvest and should be kept in insulated boxes or a cooler at around 0ºC until use.

- Bud twigs are cut into segments using a saw or pruners.
  - Each segment is about 3.5 cm long and containing a single bud (alternate arrangement) or 2 buds (opposite arrangement).
  - In some cases, dormant buds may be treated with an osmoticum (such as 5 M sucrose) for 96 h prior to desiccation to increase freeze resistance and cryo-survival of dormant buds (Tanner *et al.*, 2021b).
- Bud segments are placed on a rack in a 0ºC cooler at low humidity to dry to a moisture content of 25-30% on a fresh weight basis (fwb).

✓ **The moisture content of the bud sections is calculated prior to packaging.**

- The initial moisture content of 10 nodal sections is calculated by measuring the fresh weight (FW) and dry weight (DW).
- Dry weight is taken after sections, placed in an oven-safe tray are dried for three days in an oven at 100ºC.
- After weights are established, the following equation is used to calculate the initial moisture content: (FW-DW)/FW x 100% (on a fresh weight basis).
- The total fresh weight of the remaining nodal sections is measured, and the desired ending weight is calculated based on a desired moisture content of 25-30% (fresh weight basis).

✓ **After moisture adjustment, bud sections are packaged into polyolefin tubes (10 buds per tube), sealed, and cooled to -30ºC at rates ranging from 1ºC per hour to 5ºC per day using a programmable freezer, depending on the procedure.**

[17] See section 5 on long-term storage in liquid nitrogen.

✓ **Tubes containing buds are held at -30ºC overnight and then placed into the vapor phase of liquid nitrogen (LNV).**

- For dormant bud segments, it is recommended to cryopreserve 17 tubes for each accession, with 2 tubes to be warmed for viability assessment after LN exposure, and the remaining 15 tubes kept in long-term storage (Volk *et al.*, 2017).

4.5 Cryopreservation of shoot tips

Vegetative shoot tips (with meristems) may be cryopreserved for genebank accessions whereby the precise genotype (i.e. cultivar) must be maintained. This method is most often used for species/crops that are vegetatively propagated and are often maintained as plants in the field, greenhouse/screenhouse, or *in vitro* in the genebank. In addition, shoot tip cryopreservation may be used to cryopreserve wild species that do not have sufficient seeds, can't be cryopreserved by dormant buds, or has recalcitrant seeds for which embryos cannot be successfully cryopreserved.

Cryopreservation of shoot tips is more labour intensive than dormant buds, but it is effective for a wide range of species and can be carried out throughout the year. No single protocol is readily applicable to a wide range of plant species. A benefit of using shoot tips of *in vitro* material for cryopreservation is that the plants can undergo a virus elimination process before being cryopreserved, allowing for direct usage and dissemination after the plants are recovered. The preferred cryopreservation method will vary with the crop and is dependent upon the equipment available and technical skills. There are numerous steps in the shoot tip cryopreservation procedure that must be optimized for successful processing. These steps are described in detail by Bettoni, Bonnart and Volk (2021), Bettoni, Chen and Volk (2024), Nagel *et al.* (2024), and Vollmer *et al.* (2021) and summarized briefly below (see Volk, 2020).

✓ ***Ex vitro* or *in vitro* source plant materials are selected for use in shoot tip cryopreservation.**

Adequate quantities of shoot tips are needed for cryopreservation. In some cases, sufficient quantities can be acquired directly from plant materials collected from the field or greenhouse/screenhouse. This saves the labour involved in introducing plants into tissue culture and multiplication—which can save months of time. In many cases, it is not possible to successfully cryopreserve directly from *ex vitro* plants and so *in vitro* plants must first be established.

a) Shoot tips derived from field or greenhouse/screenhouse grown plants

Excision of shoot tips from field, greenhouse/screenhouse or growth chamber grown materials may be more efficient than introducing all plant materials into tissue culture prior to use. The use of shoot tips from these sources may be seasonal, depending on the plant physiology, dormancy, etc. of the source material (Ellis *et al.*, 2006; Volk *et al.*, 2015). Examples of protocols whereby shoot tips are derived from field plants: garlic (Ellis *et al.*, 2006); screenhouse plants: citrus (Volk *et al.*, 2015); growth chamber plants: *Vitis* (Bettoni *et al.*, 2019a, b).

- A sufficient quantity of shoot tips within *ex vitro* plant materials are obtained. Shoots, cloves, or other propagative materials (containing shoot tips) are acquired and trimmed to a size that achieves successful surface sterilization.
- Explants are surface sterilized to remove all micro-organisms prior to cryopreservation.
- For citrus shoot tip cryopreservation directly from screenhouse-grown budwood, nodal sections are surface sterilized with 70% isopropanol for 3 minutes followed by 3 rinses with sterile water. After transfer to a laminar flow hood, the nodal sections are treated with 10% commercial bleach (0.825% sodium hypochlorite final concentration) and a drop of Tween 20 (1 drop per 100 mL water) for 10 minutes, followed by three rinses with sterile distilled water (Volk *et al.*, 2020).

- In some cases, nodal sections taken from *ex vitro* source plants are cultured *in vitro* for a few weeks to produce uniform apical shoot tips for excision (Bettoni *et al.*, 2021).

b) Shoot tips derived from *in vitro* cultures

- Excision of shoot tips from *in vitro* plants requires that the accession is first be introduced and multiplied *in vitro* (see FAO, 2022c).
- *In vitro* culture systems should be optimized to ensure that cultures are clean and that optimal culture media formulation and growth conditions have been established prior to cryopreservation.
- Tissue culture plants are multiplied to produce a sufficient number of shoot tips for cryopreservation.
- It is important to use cultures that have been introduced into tissue culture relatively recently to reduce the possibility of somaclonal variation.
- *In vitro* plants must be free of endophytes and ideally sanitized so that only pathogen-free plant materials are cryopreserved. Stresses encountered during the cryopreservation process tend to exacerbate the deleterious effects of endophytes in cultured plants (Volk *et al.*, 2022).
- *In vitro* source plants may be pretreated with hormones, reduced temperatures, antioxidants, or salicylic acid prior to shoot tip excision to improve the success of the cryopreservation procedure (Bettoni, Bonnart and Volk, 2021).
- In some cases, nodal sections are cultured from the *in vitro* source plants to produce uniform shoot tips for excision (Vollmer *et al.*, 2021).

✓ **Shoot tips are excised from either surface sterilized source material or sterile *in vitro* cultures in a laminar flow hood that provides sterile conditions, using a binocular microscope.**

Excised shoot tips are often 0.8-1.5 mm in length. Demonstrations of the process are shown as videos demonstrated in the ebook "Training in plant genetic resources: Cryopreservation of clonal propagules" (Volk, 2020) as well as in a training video published by the International Potato Center (CIP, 2023).

- Excised shoot tips may be precultured for hours to days prior to cryoprotection. Shoot tips are usually precultured with elevated sucrose concentration and sometimes with other additives such as salicylic acid, glycerol, and/or dimethylsulfoxide (DMSO), on solid, semi-solid, or liquid medium, at reduced temperatures. All conditions are optimized to increase the success of the cryopreservation procedure (Bettoni, Bonnart and Volk, 2021).
- Excision of propagules can trigger the generation of reactive oxygen species (ROS), which are potentially harmful (Roach *et al.*, 2008). To counteract any negative effect of ROS production[18], freshly isolated shoot tips can be bathed in a solution of vitamin antioxidant (vitamin C) or non-vitamin antioxidant (e.g. glutathione) or elicitors of defense-related proteins in plants (e.g. salicylic acid) or anti-stress compounds (e.g., glycine betaine). The optimum concentration (mM) is determined for each propagule and plant species (Kim and Popova, 2023; Uchendu *et al.*, 2010 a, b).

✓ **Excised, precultured shoot tips are exposed to LN**
Cryoprotection treatments remove freezable water and usually introduce permeable solutes that allow undifferentiated cells in shoot tips to survive LN temperatures. Numerous options are available that use either physical drying or chemical treatments (Bettoni, Bonnart and Volk, 2021; Engelmann, 2004; Panis *et al.*, 2020; Reed, 2008).

- Encapsulation-dehydration methods place excised shoot tips in calcium alginate beads that are then treated with solutions (often sucrose) of increasing concentration. Beads containing

[18] Standard 6.5.3

shoot tips are treated with sucrose-enriched medium at either fixed or increasing concentrations for several hours or day and then physically dehydrated by either air-drying in the air current of a laminar flow hood or with silica gel to an optimum moisture content (dependent upon procedure and plant species) prior to placing beads in a vial and plunging the vial into LN (Bettoni, Bonnart and Volk, 2021).

- Vitrification methods rely on chemical cryoprotectant treatments, often using plant vitrification solutions (often Plant Vitrification Solution 2 (PVS2; Sakai *et al.*, 1990) or Plant Vitrification Solution 3 (PVS3; Nishizawa *et al.*, 1993) for an optimum time and temperature determined for the material to balance osmotic dehydration and chemical toxicity before deep cooling. The duration of cryoprotectant treatment is dependent on the taxa, physiology of the source materials, pretreatments, shoot tip size, as well as the osmoprotection and cryopreservation protocol. After preculture, shoot tips undergo osmoprotection in loading solution, which usually applies glycerol and sucrose (such as 2 M glycerol + 0.4 M sucrose) to induce tolerance and mitigate the osmotic shock to the subsequent vitrification treatment at either room temperature or 0°C. Shoot tips are either placed in cryoprotectant solution in vials (vitrification method) or in a droplet of cryoprotectant on a sterile foil strip (droplet-vitrification method) or attached to cryoplates that are submerged in cryoprotectant solution (V-cryoplate) and then plunged into LN. For droplet–vitrification and cryoplate methods, the frozen foil strip or plates, respectively, are then inserted into a pre-cooled cryovial (Bettoni, Bonnart and Volk, 2021).
- Encapsulation-vitrification methods combine encapsulation-dehydration and vitrification procedures; shoot tips are encapsulated within calcium alginate beads which are osmoprotected and treated with cryoprotectant solutions prior to LN exposure (Bettoni, Bonnart and Volk, 2021).
- Other methods, such as 2-step cooling, and D-cryoplate, are also described in the literature (Reed, 2008; Bettoni, Bonnart and Volk, 2021; Niino *et al.*, 2019; Zhang *et al.*, 2023).
- In general, vitrification protocols are faster than encapsulation-dehydration and 2-step cooling methods. However, shoot tips must be handled carefully and it is critical to have very precise control of exposure time in cryoprotectant solutions.
- When conserving a wide range of genotypes that respond differently to a protocol, modifications to the methods, or multiple different methods might be needed (Bettoni, Bonnart and Volk, 2021; Zhang *et al.*, 2023).

✓ **The quantity of cryopreserved shoot tips per accession varies depending on the desired number of materials that will be placed in cryostorage, infrastructure, availability of technical staff, material availability, and preservation goals.**

The total number of stored propagules should be high enough to be able to produce sufficient quantities of living plants to meet any future needs. For example, the National Laboratory for Genetic Resource Preservation (NLGRP), the Indian Council of Agricultural Research-National Bureau of Plant Genetic Resources (ICAR-NBPGR) and the International Potato Center (CIP), 10 cryopreserved shoot tips are placed into 1 mL cryovials). At NLGRP, 17 vials with 10-shoot tips each are cryopreserved for each accession, with 2 warmed immediately for regrowth assessment and 15 remaining in long-term storage. At CIP, 12 vials with 10-shoot tips each are cryopreserved for each accession, with 2 warmed immediately for regrowth assessment and 10 remaining in long-term storage. For some crops at ICAR-NBPGR, 20 vials are stored containing for 200 explants. For seeds, the number of stored propagules depends on the seed size, space available, seedlot heterogeneity, and the expected number needed for viability assessments and for return to the active site.

✓ **The number of vials is determined by the number of thaw events deemed necessary for that accession.**

Protocols at NLGRP aim to cryopreserve 17 cryovials for each accession (total of 170 shoot tips), with 2 cryovials warmed for viability assessment after LN exposure, and the remaining 15 vials kept in long-term storage (Volk *et al.*, 2017). At, CIP and ICAR-NBPGR, 12 vials with 10-shoot

tips each are cryopreserved for each accession, with 2 warmed immediately for regrowth assessment and 10 remaining in long-term storage. Other laboratories cryopreserve enough propagules to ensure with high confidence that a desired number of plants can be regenerated (Panis, 2009).

- Probabilistic viability calculations are effective tools that have been developed to assist in determining the predicted number of shoot tips that should be cryopreserved, based on the number of explants initially processed and the level of regrowth after LN exposure (Dussert *et al.*, 2003; Volk *et al.*, 2017).

4.6 Cryopreservation of desiccation-tolerant pollen

Pollen may be cryopreserved, although this propagule type is not generally considered a back-up of genebank accessions that are maintained as plants. Pollen cryopreservation is a vital tool for conservation of dioecious species to conserve male individuals. Preserved pollen may be particularly valuable for breeding programs for making crosses. Methods should be tested for the species of interest, ideally ensuring that cryopreserved pollen can be employed for successful crosses (see Rajasekharan and Rohini, 2023).

✓ **Upon receipt, pollen is equilibrated either in a temperature and humidity-controlled room or over saturated salt solutions to adjust the moisture content.**

- Pollen can be equilibrated at room temperature and RH between 30 and 50% overnight over saturated salt solutions of calcium nitrate (RH 46% at 22ºC; Araújo de Oliveira et al., 2021) or magnesium chloride (RH 33% at 20ºC; Nebot et al., 2021) or in precise dissolutions of LiCl (Gold and Hay, 2014; Nebot et al., 2021).

✓ **Moisture contents of the equilibrated pollen are measured and recorded.**

- Moisture contents are determined by measuring the FW, moisture adjusted, and DW (4 days at 90ºC) of a small aliquot (1-5 mg) of pollen using a precision balance. Percent moisture content equations for calculations on a fresh weight basis [(FW-DW)/FW]*100 and dry weight basis [(FW-DW)/DW]*100. For example, date palm pollen moisture content was about 6.2% fresh weight basis after adjustment over saturated salts of calcium nitrate (Araújo de Oliveira *et al.*, 2021).

✓ **After moisture equilibration, pollen is aliquoted into cryovials.**

- The number of vials prepared for each cryopreserved accession is dependent upon the quantity of pollen available and the number of pollination events desired (i.e. 0.5 mL aliquot for Rosaceae pollen, 2 mL aliquot for walnut and pecan pollen, 4 mL aliquot for date palm pollen).
- It is recommended that 15 cryovials are prepared for each genebank accession cryopreserved as pollen (G. Volk, personal communication).
- If desired, pollen viability levels may be assessed prior to and post- cryo-exposure to ensure that healthy pollen was received and processed successfully (see section 6).

✓ **Pollen cryovials are placed into the LNV for long-term storage.**

Table 5. Summary of the workflow and activities for cryopreservation of diverse propagule types.

The methodology to prepare samples for cryostorage is determined based on the species, propagule type, and conditions of the source material.	- Optimize appropriate species-specific protocols for the preparation for all propagule types that will be cryopreserved.
Best practices are utilized during sample preparation prior to packaging.	- Only introduce viable and disease -free propagules into cryopreservation. - Use clean and sterile containers for cryostorage. - Use *in vitro* source plants that have not been subcultured for extended lengths of time as donors of propagules for cryopreservation. - Ensure that adequate quantities of propagules are cryopreserved to ensure that genetic shifts do not occur if there is a decline in viability during storage. - Protect samples from lethal ice formation by air drying or application of cryoprotectants.
Samples are packaged and labelled affixed to package.	- Choose vessels and materials suitable for storage in LN/LNV based on propagule type, size and protocol used. - Use labels that will remain adhered to the package at LN temperatures.
Samples are prepared for cryostorage in LN/LNV.	- Cool samples fast enough to avoid intracellular ice formation during the cooling or warming process. - Use a temporary holding cryotank until samples deemed ready for long-term storage.
Samples are placed into LN/ LNV for long-term storage.	- Unlock the cryo tank and very gently lift the lid to avoid fogging.
Record, validate and upload all cryopreservation data, including associated metadata.	

5. Long-term storage in liquid nitrogen

The genebank is recommended to have documented policies and/or procedures, as applicable, for the long-term storage of germplasm in LN, including labelling, sample containers specifically designed for LN storage, cryotank selection and maintenance, and whether materials will be stored in the liquid or vapor phase of liquid nitrogen (LNV).[19]

Cryobanks must determine if cryopreserved materials will be stored in the vapor phase (LNV) or liquid phase of LN. This decision is based on a number of factors (see Table 6). Cryobanks may store propagules in either the liquid and/or the vapor phase of LN. Each has some advantages and disadvantages.

Table 6. Comparison of liquid and vapor phase liquid nitrogen storage.

Liquid nitrogen (liquid phase) storage	**Liquid nitrogen vapor (LNV) storage**
Consistent temperatures of –196 °C.	Sample temperature should be confirmed to remain lower than -160 °C if LNV is used. Samples may experience a temperature gradient depending on where in the tank the material is stored (it is colder closer to the LN in the lower part of the tank). Samples at the top of the tank may undergo temperature fluctuations when the lid is removed and vapor is cleared to read sample labels. Sample temperatures may fluctuate as the LN level drops between refilling events.
Increased temperature buffering due to the presence of LN on/near the samples.	Higher risk of temperature fluctuations during sample handling (lifting towers from LNV) for retrieval or inventories.
More difficult to view and retrieve samples.	Easier handling to and ease of locating and retrieving samples within the cryotank.
Possibility of microbes or other contaminant movement between containers through the liquid phase.	Lower risk of contaminant movement between containers.
Risk of cryovial caps loosening as samples jostle in the tank, and possible loss of adhered labels.	Lower risk of sample jostling during tank filling (lids come off, vials come off canes, labels come of containers etc.).
Increased safety concerns handling liquid phase (splashes, etc).	LN splashing during sample handling is less of a concern. Lower risk of explosion because the liquid phase of LN does not enter containers.

[19]See Table 7 at the end of this section for a summary of the workflow and activities for long-term storage in liquid nitrogen.

✓ **Standard operating procedures are followed for container labels to be used in LN or LNV**

- Labels should be tolerant of LN and/or LNV conditions.
- Labels should include at least the accession and inventory identifiers, date of cryopreservation. Depending upon LN storage system, tube sleeves or boxes may also be labelled with inventory information.
- Consider using barcodes to help identify if materials of interest are present in cryopreserved containers. Other alternatives include Radio Frequency Identification (RFID) tags (smart barcodes).
- Microfilm systems can be used as an option for long-term safety back-ups.

✓ **Cryotanks are selected based on storage needs and criteria**

Cryotanks are expensive equipment that could easily be in use for >25 years. Many factors must be considered when selecting a cryotank, including:

- quantity of materials to be stored
- ease of access to the materials
- tank longevity
- automation requirements

Further information can be obtained in the Best Practices Manual of the International Society for Biological and Environmental Repositories (ISBER, 2023).

✓ **Samples are be kept in the cryopreserved state during storage.**

It is important that samples remain under cryoconditions at all times to limit damage and maintain viability.

- Minimize access to inventories within cryotanks, open cryotanks as minimally as possible.
- Use best practices for crypreservation.[20]
- Monitor amount of LN in cryotanks regularly.
- Fill LN tanks as needed to ensure the quantity of LN in the tank is sufficient to maintain the required temperature.
- Measure the quantity of LN that is added to each tank during the filling process and use this information to determine if tanks are using unusually high levels of LN between tank fills. This could indicate that a cryotank is beginning to fail and must be replaced before materials are inadvertently warmed and lost.
- Ensure that ample supplies of LN are available at all times in case tanks need replenishment, particularly if temperature control is a challenge due to seasonal temperature fluctuations.

✓ **Designated technical staff are trained in safe handling procedures for LN as well as cryotank set-up and maintenance[21]**.

✓ **A reserve cryotank with LN and no samples in the cryotank room is available for use in case a tank fails and materials must be immediately moved to a new tank.**

[20] See section 4.
[21] See section 11.

Table 7. Summary of the workflow and activities for long-term storage in liquid nitrogen.

Standard operating procedures are followed for container labels to be used in LN or LNV.	- Use labels compatible to withstand LN/LNV conditions. - Ensure data on labels contain minimal information required. - Consider the use of barcodes or RFID tags.
Cryotanks are selected based on storage needs and criteria.	- Select cryotanks based on quantity of materials to be stored, ease of access to the materials, tank longevity and automation requirements.
Samples are be kept in the cryopreserved state during storage.	- Minimize access to cryotanks. - Monitor LN amounts in cryotanks regularly and fill LN tanks as needed. - Ensure sufficient supply of LN is available at all times.
Designated technical staff are trained in safe handling procedures for LN as well as cryotank set-up and maintenance.	- Ensure that all staff involved in cryopreservation are trained in best practices including safety protocols.
A reserve cryotank with LN and no samples in the cryotank room is available for use in case a tank fails and materials must be immediately moved to a new tank.	
Record, validate and upload all long-term storage data, including associated metadata.	

6. Viability/regrowth assessment, monitoring of cryopreserved propagules and post-cryopreservation quality assessment

The genebank should have a documented policy and/or procedure, as applicable, for determining the viability/regrowth monitoring system used for cryopreserved samples.[22] Ideally, the procedure should also confirm that the genetic integrity of the sample is maintained after it is retrieved from LN. Some general guidelines are also available in Benson *et al.* (2011a, b).

Viability /regrowth assessment

- ✓ **A subset of each accession that is cryopreserved[23] is assessed for viability/regrowth immediately after the cryopreservation procedure to confirm that the cryopreservation process was successful.[24]**
    - Cryopreservation success should be determined based on regrowth of the cryopreserved material, with regrowth measured by the ability to form a healthy plant or, in the case of pollen, result in a fertilization event. Pollen viability is determined by *in vitro* pollen tube growth on medium (Rajasekharan and Rohini, 2023).
    - All materials placed into cryostorage should have an initial viability/regrowth measurement for an initial baseline and to determine if the cryopreservation process was successful.

- ✓ **The methodology of viability/regrowth assessments is determined based on the species and propagule type.**

- ✓ **A subset of containers per accession are removed and warmed after cryo-exposure for regrowth assessment.**
    - The sample size will be dependent on the species and propagule type. Ideally, one or a few containers have been identified for viability assessment for easy removal. In some cases, seeds and other propagules may be removed from a container and the container returned to cryostorage. Warming procedures vary by propagule type/species/cryopreservation procedure.

- ✓ **Viability or regrowth levels are determined from the number (percentage) of healthy seedlings or plants obtained after LN exposure.**
    - Viability is calculated as the ratio of the number of propagules initially processed to the number of successfully cryopreserved propagules.
    - Reliable cryopreservation methods that result in high levels of regrowth across a wide range of genotypes are crucial to develop and implement back-up collections in LN.

- ✓ **Viability/regrowth assessments are generally performed for two replicate cryopreserved samples (i.e. vials of 10 shoot tips each).**
    - Viability/regrowth measurements are recorded for each replicate in the information management system
    - If contamination is observed during the regeneration process (particularly for *in vitro* regenerations), then an additional 1 or 2 vials may be warmed and assessed for viability. If contamination is observed in the subsequent assessments, the samples should be cryopreserved again.

[22] See Table 8 at the end of this section for a summary of the workflow and activities for viability/regrowth assessment, monitoring of cryopreserved propagules and post-cryopreservation quality assessment
[23] This is particularly necessary for embryos/embryonic axes of recalcitrant seeds, dormant buds, shoot tips, and pollen)
[24] Standard 6.5.2.

- ✓ **The regeneration threshold is set as high as possible based on species and propagule type.**
    - Due to the challenges of successful introduction into cryostorage, regeneration thresholds for germplasm maintained using other conservation methods are often much higher than what is deemed acceptable under cryopreservation.
    - At NLGRP, for example, regrowth standards are 40% regrowth levels and a total of 60 predicted viable explants in cryostorage (across all stored containers) for dormant bud and shoot tip inventories. This is calculated by multiplying the total number of shoot tips of an inventory placed into long-term storage by the regrowth percentage.

- ✓ **Viability data are stored in the genebank information management system for each cryoprocessing event.**
    - Data to consider include dates of viability monitoring and procedure (warming, media, incubation conditions), number of viable propagules, and germination/regrowth percentage. Consider the use of electronic devices to avoid transcription errors and for ease of uploading into the genebank information management system. Otherwise, the use of indelible ink (or pencil) and clear, legible writing is required when recording data. The use of barcode labels and barcode readers facilitates accession management, speeds up the process and minimizes human error.

- ✓ **The genebank information management system ideally includes automated tools to check viability and flag accessions requiring regeneration/replacement.**

6.1 Viability assessment for orthodox and intermediate seeds

- ✓ **A subset of seeds per accession are available for routine testing such that that critical accessions are not depleted.**
    - Test samples are removed and warmed for post-cryopreservation regrowth assessment using standard procedures, depending on the propagule type and the species.
    - Rewarming of orthodox seeds is achieved by placing an aliquot of seeds from the storage container in the laboratory at room temperature or by rewarming the subset of seeds within a container in a water bath at 37-40°C for 10 minutes. Seeds may then be placed in a humid container overnight to reduce the risk of imbibitional damage.
    - Seeds are surface sterilized prior to germination if microbial contamination is known to be a concern.
    - For excised embryos or embryonic axes, materials are warmed at 40°C, surface sterilized (if needed, and then plated onto regrowth medium that is optimized for *in vitro* germination and growth.

- ✓ **Seed germination testing is based on optimized and documented procedures.**
    - Guidelines for germination testing of orthodox seed are provided by FAO (2022a; section 4).
    - Guidelines for germination testing of non-orthodox seed are provided by FAO (2025).

6.2 Viability assessment for recalcitrant seeds (embryos/embryonic axes)

- ✓ **A subset of embryos/embryonic axes per accession is available for routine testing such that critical accessions are not depleted.**

- ✓ **Viability embryos/embryonic axes obtained from recalcitrant seeds is assessed through successful initiation and growth through *in vitro* culture.**
    - Post-cryopreservation embryos/embryonic axes should be warmed and treated according to

pre-determined procedures and then placed onto the species-specific medium.
- Embryos/embryonic axes should be warmed quickly by placing the cryovial at 40ºC for 2 minutes.
- Surface sterilize materials that will be initiated into *in vitro* culture if they were not surface sterilized prior to cryopreservation.[25]
- Viability is assessed as greening and normal growth is assessed by the presence of roots and shoots.
- Post-cryopreservation embryonic axes may be induced to regrow via secondary somatic embryos if the epicotyl and radicle do not grow in an organized fashion.
- Post-cryopreservation axes may be grown for days to weeks in the dark before exposure to the light.
- The medium used is modified to reduce the stimulation of callus growth from the axes culture, by adjustment of the phytohormone level (see FAO 2022c).

6.3 Viability/regrowth assessment for dormant buds

✓ **A subset of dormant buds per accession are available for regrowth assessment such that critical accessions are not depleted.**

- One or two tubes containing 10 dormant buds each are removed from LNV.
- Tubes are kept at 4ºC for up to a week and shipped as needed to be at the location for regrowth assessment, if regrowth will not be assessed at the cryobank.

✓ **The method used to assess regrowth is determined based on the species and the method that has been optimized in the performing laboratory, as well as the facilities available.**

- Dormant buds are ideally assessed for regrowth by grafting (Tanner *et al.*, 2021; Volk *et al.*, 2020)
  - Warmed bud segments are rehydrated in moist peat moss for 1-2 weeks at 4ºC.
  - Buds are grafted (bud grafting procedure) onto rootstocks (1-2 grafts per rootstock) and are considered viable when a healthy shoot grows from the grafted dormant bud.
- Alternatively, dormant bud regrowth may be assessed by direct rooting (Jenderek *et al.*, 2020).
- Alternatively, dormant bud regrowth may be assessed by forced-bud development with antimicrobial compounds (Tanner *et al.*, 2020; 2021a).
  - Buds are sprouted.
  - Ideally, dissected buds are surface sterilized and shoot tips are excised from sprouted buds and introduced into tissue culture (Tanner *et al.* 2021a).

✓ **The viability threshold is set as high as possible to ensure a maximum number of viable propagules are in cryostorage that have a high likelihood of successful regeneration.**

- Dormant bud regrowth levels are ideally set at >40% (Volk *et al.* 2017).

6.4 Viability assessment for shoot tips

The method used to assess shoot tip regrowth is based on optimized methods that have been developed for each species.

✓ **A subset of shoot tips per accession are removed and warmed for post-cryopreservation regrowth assessment.**

Common methods are as follows:

[25] Standard 6.5.4.

- Vials containing 10 shoot tips are warmed by thawing at 40ºC for 2 minutes or by directly diluting the droplets of PVS2 or other cryoprotectants into 1.2 M sucrose (unloading solution) at room temperature (Volk, 2020).
- Shoot tips are treated in 0.6 or 1.2 M sucrose for 20 minutes at room temperature to dilute cryoprotectant solutions (Vollmer *et al.*, 2019).
- Shoot tips are usually plated onto medium for regrowth assessment.
    - Multiple media may be necessary to successfully revive a plant.
    - Media are specific to species, and sometimes to accessions.
    - In some cases, no or low-light may be used prior to full-lighting conditions (Vollmer *et al.*, 2021).
    - For some systems, micrografting of shoot tips onto *in vitro* seedling rootstocks may be necessary (i.e. citrus; Volk *et al.*, 2015; Volk, Bonnart and Shepherd, 2020).
- Depending on species/protocol, regrowth measurements may be taken between 4 and 12 weeks after placement on medium.
- It is important to recover shoot tips directly, without a callus intermediate to avoid the production of somaclonal variants. Therefore, the tissue culture system, including recovery media and favorable post-thaw conditions, must be optimized for healthy shoot tip regrowth without intermediate callus formation.

✓ **The viability threshold is set as high as possible to ensure a maximum number of viable propagules are in cryostorage that have a high likelihood of successful regeneration.**

- Shoot tip regrowth levels are ideally set at ≥30% (Vollmer *et al.*, 2016) or at ≥40% (Volk *et al.*, 2017), depending on the stored sample size and pre-established probability criteria.

6.5 Viability assessment for pollen

Pollen viability is assessed after cryo-exposure. Pollen may be more short-lived in LN storage compared to other propagule types. Monitoring of pollen viability is necessary because there are few reports on pollen longevity and it may be species and protocol specific.

✓ **A subset of pollen grains per accession are removed and warmed for post-cryopreservation regrowth assessment.**

- Either an entire vial containing cryopreserved pollen or a very small aliquot of pollen is taken from a vial that remains at vapor phase LN temperature.
- Sample is warmed to room temperature for 10 minutes.
- Pollen is rehydrated in a water-saturated environment (100 % RH) for 2 hours at 22ºC in the dark.

✓ **Viability of pollen is assessed using *in vitro* germination assays.**

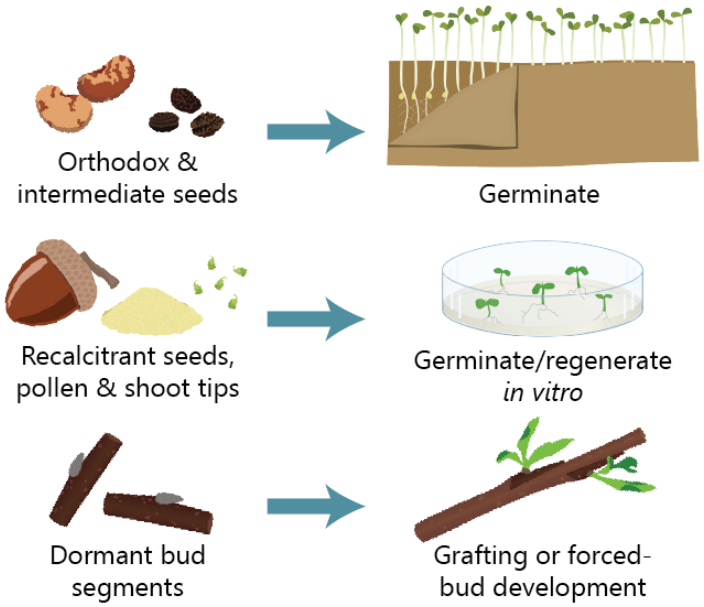
Ideally, pollen viability would be assessed by *in planta* fertilization to assess subsequent seed set, but this process is very labour intensive. *In vitro* pollen tube growth is measured by plating pollen grains onto medium and the growth of pollen tubes (germination) is used to determine viability (Rajasekharan and Rohini, 2023; Araújo de Oliveira *et al.*, 2021).

- Rehydrated pollen is plated onto medium and placed in the dark overnight. The medium and pollen tube growth system used must be optimized for each species.
- Pollen germination is measured in a compound microscope after 12-18 hours.
- Each pollen grain is considered to be germinated if the length of the pollen tube is at least the length of the diameter of the pollen grain.
- The viability of 100 pollen grains is assessed in 3 replicates (3 plates) to determine average viability levels.

✓ **The viability threshold is set as high as possible to ensure a maximum number of viable propagules are in cryostorage that have a high likelihood of successful regeneration.**

Successfully cryopreserved pollen has a viability of >20%, although samples with viability levels as low as 5% are maintained in cryostorage until they are cryopreserved with higher viability levels (G. Volk, personal communication).

Figure 7. Types of viability assessments for cryopreserved materials.



Source: Katheryn Chen, with permission

6.6 Monitoring of cryopreserved propagules

It is standard practice for seeds in long-term storage to be periodically tested for viability in a process called "monitoring". It may be feasible to sample subsets of whole seeds or pollen for viability assessments; however, samples of embryos/embryonic axes, dormant buds, and shoot tips are often stored in limited quantities. Due to the expense and difficulty of putting these collections into cryostorage, monitor testing may not be regularly or frequently performed (particularly in the early years after cryopreservation) for these propagule types. It may be more practical to cryopreserve a large quantity of material for only a few accessions, which would serve as viability checks through monitor testing for a larger set of samples of representing the same crop (Vollmer *et al.*, 2017, 2022).

✓ **A monitoring system is in place to test the periodic viability/regrowth of all cryopreserved accessions.**

- Viability monitoring aims to identify, as closely as possible, the time when viability has decreased to, or is approaching, the determined threshold for regeneration.
- Monitoring of cryopreserved seed in long-term storage may not occur as regularly as conventionally stored seed. Frequent monitoring may not be justified for explants of recalcitrant seeds, dormant buds, and shoot tips, given the high levels of labor required to reintroduce materials into long-term LN storage.

✓ **Realistic monitoring intervals are set based on propagule type**

- Setting monitoring intervals is a compromise between the need to avoid wasting propagules and resources, and the risk that valuable material may be lost if monitoring is too delayed or infrequent.
- In practice, the frequency of viability monitoring of cryopreserved materials (e.g. 10 to 25 year intervals) is often dependent upon the resources available at the genebank. Pollen may be tested more frequently, such as at 4-year intervals.

6.7 Post-cryopreservation quality assessment

The goal of any cryopreservation program is to maintain the viability and quality of materials after LN storage. Regenerated plants after cryostorage should have identical phenotypes and genotypes as the source materials. Benson *et al.* (2011a) are a source of information about physical and genotypic stability after cryopreservation.

For populations, such as cryopreserved seed or pollen, genetic shifts may occur if some genotypes within the population exhibit a decline in viability or regeneration capacity. As a result, adequate quantities of propagules should be provided for regeneration efforts.[26]

- ✓ **Quality assessment is based on the regeneration of whole plants (normal phenotype and true-to-type) from regenerated materials.**

  These assessments can be performed based on genebank policies for phenotypic evaluations (see other guides FAO, 2022a, b, c).

- ✓ **Genetic characterization data may be collected for accessions recovered after cryopreserved to ensure that they are true-to-type after cryostorage.**

  Genetic characterization through the use of genotypic markers or genome sequencing may document significant issues (such as mixed-up samples due to labelling or processing errors) but may not identify single point mutations in the genome. It is critical to ensure not only the viability and quality of the preserved plants, but also the true-to-type status of the regenerants.

  Most of the cryopreservation procedures make use of tissue culture to some extent, therefore the *in vitro* tissue culture system must be treated as an integral part of the cryopreservation procedure; non-optimized *in vitro* tissue culture systems may be prone to somaclonal and (epi)genetic variations in regenerants (Bettoni, Bonnart and Volk, 2021; Wang *et al.,* 2021). In addition, the application of antioxidants during the cryopreservation and/or regrowth procedures may reduce oxidative stress and improve epigenetic integrity, metabolic stability, and field performance (Ren *et al.,* 2021).

- ✓ **Cryostored materials that are processed with the intention of preserving associated microbes are tested to ensure that the associated microbes remain in the regenerated materials.**

  In some cases, cryopreservation of shoot tips may result in a change in the viral, viroid, and/or bacterial status of the source materials, an activity known as “cryotherapy” (Zhao *et al.*, 2019). Cryotherapy is not covered in this guide, for detailed information see Wang *et al.* (2022) and Bettoni and Volk (2024). Shoot tip cryotherapy relies on the differential survival of infected versus pathogen-free cells after LN exposure. The pathogen location and the differential survival of cells as a result of cryoexposure are key to the success of cryotherapy. When shoot tips are briefly exposed to LN, the meristem cells, which contain either low virus titres or are free of virus infection, survive while the vacuolated and differentiated cells that can harbor pathogens usually do not survive. As a result, healthy and disease-free plants can be regenerated. Improvements in pathogen eradication, especially those diseases that may infect the meristematic cells, have been achieved using methods that combine thermotherapy and/or chemotherapy with shoot tip cryotherapy (Bettoni *et al.*, 2024; Wang *et al.*, 2022).

[26] See section 4.

Table 8. Summary of the workflow and activities for viability/regrowth assessment, monitoring of cryopreserved propagules and post-cryopreservation quality assessment

A subset of each accession that is cryopreserved is assessed for viability/regrowth immediately after the cryopreservation procedure to confirm that the cryopreservation process was successful.	- Success should be determined based on regrowth by the ability of the cryopreserved material to form a healthy plant or, in the case of pollen, result in a fertilization event or pollen tube growth on medium.
The methodology of viability/regrowth assessments is determined based on the species and propagule type.	- Assess cryopreserved orthodox and intermediate seeds using optimized and documented protocols. - Assess cryopreserved embryos/embryonic axes through successful initiation and growth through *in vitro* culture. - Assess dormant buds by grafting onto rootstocks through healthy shoot growth. - Assess shoot tips through growth on successful plant formation in media. - Assess pollen through growth of pollen tubes on medium.
A monitoring system is in place to test the periodic viability/regrowth of all cryopreserved accessions.	- Set a realistic monitoring interval depending on the propagule type. - Monitoring is carried out as infrequently as possible, especially for explants of recalcitrant seeds, dormant buds, and shoot tips, given the high levels of labour required to reintroduce materials into long-term LN storage.
Quality assessment is based on the regeneration of whole plants (normal phenotype and true-to-type) from regenerated materials.	- Assessments based on genebank policies for phenotypic or genotypic evaluations.
Cryostored materials processed with the intention of preserving associated microbes are tested to ensure that they remain in the regenerated materials.	
Record, validate and upload all viability/regrowth assessment, monitoring of cryopreserved propagules and post-cryopreservation quality assessment data, including associated metadata.	

7. Replacement of cryopreserved inventories

The genebank is recommended to have a documented policy and/or procedure, as applicable, for replacing depleted (either by use or decline in viability) cryopreserved samples.[27]

✓ **Inventory and viability of cryopreserved materials are monitored regularly.**

Cryobank materials are depleted when materials are distributed for regeneration or when viability levels decline during cryostorage.

- Thresholds based on viability and quantity-on-hand are set within the genebank information management system to determine when inventory replacement is required.
- The genebank information management system ideally includes automated tools for checking inventory and viability, and flagging accessions requiring regeneration/replacement for materials under cryopreservation.

✓ **Cryobank inventories are regenerated or replaced when viability or quantity falls below the quantity threshold.**

✓ **Optimal regeneration/replacement practices are used to ensure sufficient and healthy material and to minimize risk to the genetic integrity of the accession**.

Regeneration practices will vary for the material being replenished. Some general practices include:

- Orthodox and intermediate seeds in cryostorage can be replaced by replenishing seeds during a seed regeneration event (see FAO, 2022a). Seeds for regeneration may be sourced from the medium- or long-term storage of the seed genebank or from cryopreserved seeds[28].
- Recalcitrant seeds in cryostorage may be replenished by either processing another set of samples from the active collection or by warming, regenerating using regrowth methods listed in section 5, and then cryopreserving materials from the regenerated stock.
- Dormant buds can be replenished by collecting new dormant buds from an accession and repeating the cryopreservation process. If the field trees are no longer available, dormant buds can be regenerated (buds are grafted onto rootstocks) from the cryopreserved samples and grown as trees and grown until sufficient dormant buds are available,[29] and then cryopreserved.[30] Alternatively, collection missions to the same site could be considered if feasible and resources are available.
- Shoot tips can be replenished by cryopreserving shoot tips collected from field, greenhouse/screenhouse, growth chamber, or *in vitro* plants. In some cases, a remaining cryo-inventory may be warmed, repropagated *in vitro*, and cryopreserved to replenish the supply. As shoot tips are clonal propagules, each of the regenerated propagules should be genetically identical.
- Pollen can be replenished by cryopreserving new pollen from the original genebank field plants.

✓ **All regeneration data, including associated metadata, are recorded, validated and uploaded to the genebank information management system.**

- Data to consider for the regeneration of seed or trees include planting and harvest dates, cultural practices used (spacing, weeding, irrigation, fertilizer, pesticide

[27] See Table 9 at the end of this section for a summary of the workflow and activities for replacement of cryopreserved inventories.
[28] Cryopreserved seeds will need to undergo the warming process (see section 5).
[29] Optimal procedures should be used for regeneration in the field (see FAO, 2022a for regeneration of seeds and FAO, 2022b for regeneration of trees and clonally propagated species)
[30] As dormant buds are clonal propagules, each of the regenerated propagules should be genetically identical.

application, etc.) and dates when implemented, number of plants harvested, yield, etc. Data for explants regenerated *in vitro* include date of subculture, date of reinitiating into culture, etc.

- Consider the use of electronic devices (i.e. barcodes) to avoid transcription errors and for ease of uploading into the genebank information management system. Otherwise, the use of indelible ink (or pencil) and clear, legible writing is required when recording data.

Table 9. Summary of the workflow and activities for replacement of cryopreserved inventories.

Inventory quantity and viability are monitored for cryopreserved materials.	-Cryobank inventories are regenerated when the quantity and/or viability drop below critical levels.
Optimal regeneration/replacement practices are used to ensure sufficient and healthy material and to minimize risk to the genetic integrity of the accession.	-Regeneration/replacement practices are specific to the propagule type and species.
Record, validate and upload all cryopreservation data, including associated metadata.	

8. Distribution

The genebank is recommended to have a documented policy and/or procedure, as applicable, for the distribution of germplasm, including the review process for checking for fulfilment of legal, phytosanitary and other regulations and requirements, and step-by-step instructions for consignment preparation, post-consignment follow-up and reporting to the Secretariat of the Treaty or a National Focal Point or other designated authority, as necessary.[31]

Nearly all genebank samples are distributed from the active collection as either seeds or plant parts such as dormant buds, cuttings, tubers, etc. Samples in the cryobank are most often only distributed back to the active site to replenish accessions that have been lost, commonly because the seed viability dropped to 0% or because the *in vitro* or field genebank plants either died or became unhealthy. In these cases, materials are warmed at the cryobank location and then shipped back to the active site. If the recipient active collection site has the necessary skills for regeneration, cryopreserved materials may also be shipped in the frozen state.

Cryopreserved safety duplicated materials may also be returned to the provider when those materials are needed at the source genebank. In this case, due to the expertise and cost required to regenerate materials after cryostorage, the cryopreserved samples are often shipped back to the original provider in a cryopreserved state. It is important that the recipient genebank has the adequate infrastructure and capacity to keep the material in a cryopreserved state during the transfer process.

✓ **Required documentation is requested and obtained.**

- Import permit regulations, which specify phytosanitary and any other import requirements, including packaging requirements, must be requested from the relevant national authority of the receiving country. Documents often required by the recipient country include a phytosanitary certificate, additional declarations, a certificate of donation, a certificate of no commercial value and an import permit.
- The genebank is encouraged to use a Standard Material Transfer Agreement (SMTA) (FAO, 2021b, c).

✓ **The distribution process and quantity of propagules distributed is dependent upon the propagule type and packaging.**

- If the recipient has the necessary skills for regeneration, cryopreserved materials may be shipped in the frozen state.
- For materials in cryovials or tubes, such as dormant buds, shoot tips or embryonic axes, a single tube of 10 explants will most often be warmed, regenerated (see section 6 above), and then distributed. This may take several months.
    - For materials reestablished though *in vitro* culture, the average quantity shipped is approximately 3–5 plantlets per accession (see FAO, 2022c for further guidance).
    - Alternatively, for dormant buds, a tube containing 10 dormant buds may be warmed, shipped, and placed in a 4-5ºC cooler and the recipient would graft onto rootstocks in 7 to 14 days.
- For orthodox and intermediate seeds, the number of seeds distributed is dependent upon seed size, viability, and availability (see FAO, 2022a for general guidance). Note that it may be possible to carefully remove some seeds and return the seed package to LN.

[31] See Table 9 at the end of this section for a summary of the workflow and activities for distribution of germplasm.

- For pollen, a vial containing a predetermined quantity of pollen can be distributed in the cryopreserved or warmed state and used for pollination immediately upon receipt.

✓ **The length of time between receipt of a request for samples and their dispatch is kept to a minimum.**

✓ **Samples are labelled carefully and are not mixed during handling.**
Samples should be correctly labelled, preferably with computer-produced labels to reduce transcription errors. Labels should be placed both outside and inside each package to ensure that the material is properly identified.

✓ **The choice of packaging material and transport allows for safe and timely delivery.**

Ensure that the material reaches the destination genebank in good condition, keeping in mind the time needed for document processing, duration of shipment, transit time and transit conditions (high temperatures and/or humidity in tropical countries).

- For specific guidance, see FAO (2022a) for distribution of seeds, FAO (2022b) for distribution of cuttings and vegetative materials, FAO (2022c) for distribution of materials maintained *in vitro* and FAO (2025) for distribution of recalcitrant seeds.
- For the shipment of materials to be distributed in the frozen state, it will be necessary to use a cryo-shipper container to keep materials at LN temperatures.

✓ **All required documentation is included inside the shipment (for the recipient) and attached to the outside of the container for the customs officials in order to guarantee smooth processing during transit and at the border of the destination country, if it is an international transfer.**[32]

Consider scanning documents and sending them by email, or sending hard copies by mail, prior to the dispatch of the germplasm. Documentation to consider include:

- data on accessions (including an itemized list with accession identification, number and/or weights of samples, and key passport data); and
- import permit, phytosanitary certificate, or customs declaration, if appropriate.

✓ **The delivery and condition of the germplasm on arrival at its destination is followed up to confirm that germplasm has reached the recipient sufficiently quickly.**

It is suggested that shipments be tracked and that the genebank follow up with the recipient regarding the status and usefulness of the distributed germplasm.

✓ **All distribution data, including associated metadata, are recorded, validated and uploaded to the genebank information management system.**

Data to consider include requester's name and address, purpose of request and request date; samples requested, samples sent and number of plantlets per sample; virus indexing method; reference to phytosanitary certificate and SMTA or MTA; and shipping log and user feedback. Consider the use of electronic devices to avoid transcription errors and for ease of uploading into the genebank information management system. Otherwise, the use of indelible ink (or pencil) and clear, legible writing is required when recording data. The use of barcode labels and barcode readers facilitates accession management, speeds up the process and minimizes human error.

[32] Standard 6.7.2.

Table 10. Summary of the workflow and activities for distribution of germplasm[33]

Distribution of germplasm	
Genebank complies with national, regional and international regulations and agreements.	-Cryobank materials held as safety duplicates for another institution are returned according to the conditions of the original agreement between the institutions.
A policy is in place for the number of propagules or vials/containers to distribute.	- Cryopreserved materials are distributed as appropriate for the materials. - In some cases, materials are repropagated to *in vitro* or field plants at the cryobank location prior to distribution.
Samples are carefully labelled and not mixed during handling.	- Use computer-produced labels to reduce transcription errors. - Place labels both inside and outside each packet, as appropriate.
Required documentation is provided to recipient and included with the shipping package.	- Include accession data (accession identification, number of samples and key passport data); import permit, phytosanitary certificate and/or customs declaration (as needed). - Send scanned documents/files in advance by email to the recipient.
Packaging material and transport allows for safe and timely delivery.	- Use of packaging and shipping guidelines/recommendations appropriate for the propagule.
Status of germplasm and condition on arrival is obtained.	- Track the shipment and follow up with the recipient.
Record, validate and upload all distribution data, including associated metadata.	

[33] Specific guidance is available for distribution of seeds (FAO, 2022a), cuttings and vegetative materials (FAO, 2022b), materials maintained *in vitro* (FAO, 2022c), and recalcitrant and intermediate seeds (FAO, 2025).

9. Safety duplication

The genebank is recommended to have a documented policy and/or procedure, as applicable, for the safety duplication of cryopreserved germplasm. This includes the review process for checking for fulfilment of legal, phytosanitary and other regulations and requirements, and step-by-step instructions for consignment preparation, post-consignment follow-up and shipment schedules.[34]

✓ **A safety duplicate sample for every original accession is stored in a secondary location, under cryopreservation and utilizing best practices.[35]**

Safety duplicates of cryopreserved inventories are deposited at a location well away from the main collection, which could be in another country. The safety duplicate location is chosen to minimize possible risks and provide the best possible conditions, taking into account the need for adequate facilities, staff and financial resources. It should be in a socio-politically and geophysically stable location. The genebank/institute hosting the safety duplicates should have adequate capability to provide appropriate cryopreserved conditions for the duplicated accessions. Selection of and clear agreement with the chosen holder of the safety duplicate is critical.

✓ **A legal agreement setting out the responsibilities of the depositing and the recipient genebanks, and the terms and conditions under which material is maintained and managed, should be in place.**
Discussions should take place with the host genebank early in the planning process on the required documentation (both for the genebank and the host country), and the applicable customs and quarantine procedures. This will help ensure timely movement of the germplasm.

✓ **The security back-up location complies with legal, phytosanitary and other regulations and requirements, and each safety duplicate sample is accompanied by relevant associated information.**

✓ **The safety duplicate is of high quality and consists of a sufficient quantity of material.**

✓ **Samples are labelled carefully and are not mixed during handling.**

It is important to ensure that samples are correctly labelled, preferably with computer-produced labels to reduce transcription errors in names and numbers.

✓ **The choice of packaging material and transport allows for safe and timely delivery.**

Ensure that the material reaches the destination back-up location in good condition, bearing in mind the time needed for document processing, duration of shipment, transit time and transit conditions (high temperatures and/or humidity in tropical countries). The use of packing and shipping guidelines/recommendations similar to those utilized for distribution of materials in the frozen state is recommended (see section 8).

✓ **Each safety duplicate sample is accompanied by relevant associated information.[36]**

It is recommended that relevant information be sent with the shipment, including an itemized list with accession identification, key passport data, total number of propagules, type of container, and import permit, phytosanitary certificate or customs declaration, if appropriate. If appropriate, include information related to cryopreservation method, storage conditions, and retrieval techniques. Consider scanning documents and sending them by email, prior to the dispatch of

[34] See Table 11 at the end of this section for a summary of the workflow and activities for safety duplication of germplasm.
[35] Standard 6.8.4.
[36] Standard 6.8.5.

the germplasm.

✓ **All safety duplication data, including associated metadata, are recorded, validated and uploaded to the genebank information management system.**

Data to consider include: the location of the safety-duplicated accessions (both facility and specific sample locations within cryotanks), samples sent and number of replicates per sample; shipping log and user feedback; and reference to legal agreement, phytosanitary certificate, etc. Consider the use of electronic devices to avoid transcription errors and for ease of uploading into the genebank information management system. The use of barcode labels and barcode readers facilitates accession management, speeds up the process and minimizes human error.

✓ **The genebank information management system is regularly reviewed and updated to ensure that any new material not duplicated in the recipient genebank is identified and prepared for safety duplication, as appropriate.**

Table 11. Summary of the workflow and activities for safety duplication of germplasm

Safety duplication	
Safety duplicated accessions are stored at a distant location.	- Consider issues like biosecurity, geopolitical situation, likelihood of natural disasters, cost. - Ensure hosting genebank/institute has good management capabilities to provide appropriate conditions for maintaining the duplicated germplasm.
Legal agreement defines responsibilities of depositing and recipient genebank.	
Genebank complies with legal, phytosanitary and other regulations.	- Request information from host genebank on the required documentation (both for the genebank and the host country), and the applicable customs and quarantine procedures.
Safety duplicates are of high quality and have a sufficient quantity of material.	- Ensure adequate quantities of cryopreserved materials are available so inventories can be duplicated at a secondary location.
Samples are labelled carefully and are not mixed during handling.	- Use computer-produced labels to reduce transcription errors. - Place labels both inside and outside each packet (as appropriate).
Packaging material and transport allows for safe and timely delivery.	- Use packaging and shipping protocols appropriate for LN transport.
Ensure safety duplicates are accompanied by relevant documentation.	- Include accession data (accession identification, number of samples and key passport data); import permit, phytosanitary certificate and/or customs declaration. - Send scanned documents in advance by email to the recipient.
Record, validate and upload all safety duplication data, including associated metadata.	

10. Documentation

The genebank is recommended have a documented policy and/or procedure, as applicable, for managing genebank data and information, including data-sharing guidelines.[37]

✓ **A genebank information management system is developed specifically for the genebank or one of the several systems available is used/adapted.**

- There is a need to focus on the future use of the cryopreserved material, ensuring predictability and reliability.
- The genebank information system is ideally designed to manage all the data and information generated relating to all aspects of cryopreservation
- GRIN-Global has been developed by USDA-ARS, the Global Crop Diversity Trust and Bioversity International to enable genebanks to store and manage information associated with plant genetic resources, and is freely available (GRIN-Global, 2021). More recently GRIN-Global Community Edition (GGCE; Global Crop Diversity Trust, 2024) has been made available.

✓ **International data standards are adopted to provide consistency in data shared among different information systems and programs.**

✓ **Data for cryopreserved inventories of genebank accessions should be aligned with the associated information for the source seedlot, plant or samples maintained *in vitro* if a corresponding seedlot or field plant is maintained in the genebank.**

✓ **Mobile devices are used to capture data, if possible.**

The use of barcoding facilitates all aspects of genebank management, especially documentation.

✓ **Data recorded on paper are digitalized and measures are put in place to check hand-written and electronic data entries for transcription errors.**

✓ **All data and information generated relating to all aspects of conservation and use of germplasm, including images and metadata, are validated and uploaded to the genebank information management system.[38]**

Having trained staff responsible for data recording and data entry in close collaboration with documentation officers and germplasm collection curators supports quality control. Validation of data by genebank curators and documentation officers before being uploaded into the genebank information management system is recommended.

✓ **Data are publicly available in a search-query database, as appropriate.**

Inventory information for cryopreserved inventories that serve as a back-up of the genebank's active inventories may not necessarily be publicly available. Information for the active inventories in the genebank collection is often publicly available.

✓ **Data are duplicated (backed-up) at regular intervals and stored at a remote site and/or in the cloud to guard against loss from fire, computer failure, data breach, etc.**

[37] See Table 12 at the end of this section for a summary of the workflow and activities for documentation.
[38] Standard 6.6.3.

Table 12. Summary of the workflow and activities for documentation

Documentation	
A suitably designed genebank information management system is used	

International data standards are adopted for consistency in data sharing.	- Data should be aligned with the associated information for the materials maintained in the active collection.
	  - Use FAO/Bioversity Multi-Crop Passport Descriptors.
	  - Consider using Digital Object Identifiers (DOIs).
	- Ensure all data are kept up to date.

Mobile devices are used to capture data, if possible.	- Use barcoding to facilitate accession management.
Data recorded on paper are digitalized.	- Check handwritten and electronic data entries for transcription errors.
Collection inventory data are regularly updated.	- Use built-in automated tools to check inventory and viability, and flag accessions requiring regeneration.
Viability/regrowth data are recorded.	
Germplasm orders, distribution information and user feedback are documented.	
Data are publicly available, possibly though a search–query database.	- Ensure that primary site inventory data are available for accessions, noting that cryopreserved inventory may not be publicly available.
Data are duplicated (backed-up) at regular intervals and stored at a remote site and/or in the cloud for security reasons.	

11. Personnel, security and safety

It is recommended that the genebank have a strategy in place for personnel and security, including a succession plan; a corresponding budget must be allocated and reviewed regularly.[39] Staff training for technical skills as well as for safety are particularly important within cryobanks.

11.1 Personnel

✓ **The genebank has a human resources plan with appropriate annual budget allocation, and staff have the critical knowledge, skills, experience and qualifications needed to implement all genebank tasks effectively and efficiently.**

Successful genebank management requires well-trained staff with clearly defined responsibilities for accession management.[40] The following practices should be considered:

- ensuring that the genebank manager and those staff carrying out specific tasks regularly review and update SOPs, as applicable.
- ensuring that curators and technical support staff have knowledge and skills in agriculture, horticulture and taxonomy of cultivated plants and their wild relatives.
- having access to disciplinary and technical specialists in a range of subject areas, such as taxonomy, physiology, phytopathology, breeding and population genetics, cryobiology and tissue culture.
- holding regular on-the-job training sessions and, if possible, ensuring that staff can attend training opportunities at regular intervals to keep up to date with recent developments.
- rotating tasks to make work as varied as possible and involving all staff (where possible) in meetings and discussions as well as obtaining experience in different methodologies in the conservation process as applicable.
- retaining competent staff by providing recognition and rewards for excellent performance.
- information about training programs and/or certificates may be available through some institutions such as Royal Botanic Gardens Kew, ISBER, CGIAR, etc.

11.2 Security

✓ **Genebank facilities are secure from intrusion** (Benson *et al.*, 2011b)

- Secure entry for authorized personnel (particularly for access to the cryobank) is needed
- Facility security should include cameras and recording devices to capture information about any unwelcome intruders

✓ **Risks associated with staffing are included in the risk identification, analysis and management.**

Secure conservation depends on accurate assessment and appropriate management of risks (see Annex). Therefore, all genebanks should establish and implement risk management strategies that address the physical and biological risks in the everyday environment to which the staff, collections and related information are exposed.

✓ **A risk management strategy is in place.**

A genebank is recommended to have a documented risk management strategy in place that includes measures for dealing with power cuts, fire, flooding, earthquakes, war and civil strife.[41] This strategy and an accompanying action plan should be regularly reviewed and updated to take changing circumstances and new technologies into account.

[39] See Table 13 at the end of this section for a summary of the workflow and activities for personnel and security.
[40] Standard 6.8.3.
[41] Standard 6.8.1.

A risk management strategy has the following components (SGRP-CGIAR, 2010):

- Communication and consultation: ensure that all those who will be involved in implementing a risk management system are oriented in the concepts, methodology, terminology, documentation requirements and decision-making processes of the system.
- Establishing the context: consider the objectives/activities/tasks of the genebank, the environment in which the activities operate, and the stakeholders.
- Risk identification: carry out an inventory of relevant risks to the genebank operations.
- Risk analysis: assess the potential impact (or consequence) of the identified risks and their likelihood (probability).
- Risk evaluation: determine the level of risk that is acceptable.
- Risk treatment: identify actions that need to be undertaken in order to prepare for those risks for which the current total risk rating is considered unacceptable, giving top priority to the highest assessed residual risks.
- Monitoring and review: analyze the risk management system and assess whether changes to the system are needed. Responsibilities for monitoring and review should be clearly defined and documented.

✓ **It is recommended that all genebank staff be made aware of the details of the risk management strategy and have a clear understanding of responsibilities for implementing and monitoring the strategy and action plan.**

11.3 Safety

✓ **A staff member with responsibility for occupational safety and health (OSH) in the genebank is appointed and receives training in OSH.**

OSH deals with all aspects of health and safety in the workplace and has a strong focus on primary prevention of hazards.[42] Most countries will have an OSH policy. The International Labour Organization (ILO, 2021) provides country profiles on OSH.

✓ **All staff are aware of OSH requirements and are kept up to date regarding any changes.**

✓ **All staff are routinely trained in safe operating procedures and equipment use relating to liquid nitrogen and sample handling.**

It is recommended that all genebank staff be made aware of the details of the risk management strategy and have a clear understanding of responsibilities for implementing and monitoring the strategy and action plan. Best practices to consider include:

- ensuring that OSH rules are visible in the more risk-prone areas of the genebank;
- instructing staff in the correct and safe use of equipment with regular training provided in health and safety in field, greenhouse/screenhouse and laboratory environments;
- choosing appropriate and nationally approved agrochemicals to reduce risk; and
- providing properly functioning protective equipment and clothing, as required by OSH, and ensuring that they are regularly checked and used as expected. The OSH officer is responsible for the upkeep of safety equipment.

✓ **Rooms with cryotanks should have safety measures in place to protect air quality.**

If liquid nitrogen spills or is released into the atmosphere, it expands to nitrogen gas which may displace the oxygen in the room, creating an asphyxiation risk. It is therefore critical to:

- Ensure rooms are adequately ventilated.

[42] Standard 6.8.2.

- Install oxygen alarms that sound if the oxygen level drops below 19.5%. The alarm may also activate fans to ventilate the room.
- Make personal data logging oxygen monitors available for employee use, particularly if they will be in the room or handling liquid nitrogen for extended periods of time.
- All personnel must be trained in use of the required safety and LN handling equipment.
- Limit access to cryobank storage facility to trained and necessary personnel.

✓ **Liquid nitrogen handling requires appropriate personal protective equipment to protect personnel.**

LN is -196ºC and skin contact can cause severe cold burns and thus the following safety precautions should be followed.

- Required clothing includes a labcoat, long pants, and closed-toe shoes.
- Remove all items from the labcoat top pockets to ensure glasses, pens, etc. do not fall into the cryotank.
- Safety equipment includes cryo-gloves and safety glasses/face shield when interacting with LN or cryotanks.

✓ **Retrieval of samples from cryotanks requires the use of safety equipment and appropriate tools.**

- Human faces should never be closer than 10 cm from the top of the tank opening.
- Platforms or storage devices in the cryotank may need to be moved by using safe operating procedures
- Gloves, tongs, or other tools may be needed to retrieve specific boxes or canes from the tank.
- Materials within the tank must not be warmed when the tank is open and material handling takes place.
- Vacuums or other devices may be necessary to clear the vapor from the tank to visualize the sample labels.
- Barcode readers may be used to read and identify container labels.

✓ **Ensure that the specific safety measures for cryobanks are followed.**

Best practices include:

- ensuring that personnel are trained in safe handling of LN, and the use of appropriate personal protective equipment (PPE);
- PPE are particularly important when working with materials that are in LN or LNV. LN may enter the storage container within the tank and could cause an explosion when the sample warms and the LN enters the gas phase.
- using appropriate ventilation to ensure a safe environment;
- installing an oxygen monitoring system to ensure that oxygen levels do not drop below safe levels for human presence as a result of LN vaporization;
- using an emergency alert systems when personnel are interacting with LN tanks, particularly if doing so alone.

Table 13. Summary of the workflow and activities for personnel and security

Personnel and security	
Human resources plan and appropriate annual budget allocation in place.	- Ensure necessary staff skills. - Conduct regular staff training (on-the-job and external). - Rotate tasks to make work more varied and interesting. - Retain staff by providing recognition and incentives. - Ensure that a staff succession plan is in place.
Risks associated with staffing are included in the risk identification, analysis and management.	
Risk management strategy in place.	- Communication and consultation.
	- Establishing the context.
	- Risk identification.
	- Risk analysis.
	- Risk evaluation.
	- Risk treatment.
	- Monitoring and review.
Staff member(s) appointed and trained in overseeing occupational safety and health.	- Ensure all staff are aware and trained in occupational safety and health.
Individual risks managed.	Risks to Staff - Take health and safety of staff and environment into consideration. - Choose appropriate and approved agrochemicals. - Ensure proper conditions and ventilation. - Provide protective equipment and clothing and ensure its use.
	Risk to Collection - Develop a risk management plan that includes mitigation and response. contingencies for all potential risks to the physical collection.
Record, validate and upload all personnel, security and safety data, including associated metadata.	

12. Infrastructure and equipment

This section considers the suggested infrastructure and equipment for handling germplasm in a cryobank (Table 14). Genebanks handling materials (such as non-orthodox seeds, dormant buds, shoot tips, and pollen) for cryopreservation are generally equipped with: (a) basic tissue culture equipment (autoclave, laminar flow hood, dishwasher, dissecting microscope, sterilization equipment, fine forceps and scalpels), growth rooms and support facilities (Benson *et al.*, 2011b; FAO, 2022c); (b) specialized storage equipment, such as incubators and acclimatizing chambers; (c) microscopes and analytical and molecular equipment for germplasm authentication and performance and genetic integrity testing; (d) drying ovens and balances for obtaining weights: (e) vials, polyolefin tubes, and aluminum envelopes for storage and sealing equipment for envelopes and polyolefin tubes; (f) cryotanks for storage, LN holding tanks, source tanks for LN, dewars for cryoprocessing of samples in the hood and for transport, controlled-rate coolers (if preserving dormant buds), and (g) safety equipment, such as alarms and smoke detectors, gloves and face shields, emergency personal monitors in case help must be called while in the LN areas.

Factors that should be considered if designing or modifying genebank facilities include: (a) function of the facility (cryopreservation); (b) projected throughput and number of accessions for storage; (c) expected distribution rates; (d) local climate, of particular importance in the tropics because of potential contamination issues; and (e) number of trained staff. Note, due to the unique properties of cryobanks, it may be easier to design and build a new cryobank than retrofit existing laboratory space.

An important rule to remember is that operations and workspace design should be planned so that germplasm and materials do not become contaminated, lost or misplaced. Physical delineation of clean and dirty areas, with samples progressing one-way through increasing levels of cleanliness and security is one way in which contamination and workflow can be controlled.

A reliable source of LN is critical to the success of a cryobank. LN may either be purchased or generated locally (on site) with a LN generator. For purchased LN, the source must be reasonably priced and routinely available. For LN that is generated, facilities staff must perform all necessary maintenance to ensure that the equipment is always functioning properly.

Table 14. General infrastructure and equipment recommended for a cryobank

General needs
Office space and supplies; computers, printers and accessories; label printers, climate data loggers; mobile devices for electronic data recording and barcode readers; access to scientific and technical literature; internet access.
Acquisition
Collecting equipment including cloth and/or paper bags, labels (ideally barcoded), hand lenses, scissors, secateurs, tarpaulins, packaging materials, herbarium presses¸ simple desiccation drier. Collecting data sheets or mobile devices for electronic data recording, GPS or altimeter.
Cryopreservation of germplasm
General: cryo gloves, labcoats, face shields, cryotank, dewars, LN supply tanks, oxygen monitoring system. Orthodox and intermediate seeds: conditions to adjust moisture content, weigh seeds, count seeds, package seeds, tube/container sealer, label containers.

Dormant buds: conditions to dry bud sections, bandsaw to cut buds, balance to weigh buds, oven to dry bud sections to calculate moisture content, tubes for buds, tube sealer, controlled rate cooler, cryoboxes and labels. Shoot tips: sterilization supplies (if not sources from *in vitro* culture), forceps, scalpels and supplies for tissue culture, autoclave, media making supplies, filter sterilization system for cryoprotectant solutions, dishwasher, laminar flow hood, dissecting microscope, small dewars, foil strips/cryovials, labels on cryovials, cryocanes/boxes with labels, sleeves for cryocanes with labels (depending on box/rack system for cryotank), culture vessels. Pollen: moisture equilibration conditions, sieves, packaging (correct vial size), labels for vials, cryoboxes (labeled).
Viability/regrowth assessments and monitoring of cryopreserved propagules & regeneration/replacement of cryopreserved inventories
For orthodox & intermediate seeds: seed germination supplies, blotter paper/paper towels, incubator for growth. For shoot tips & recalcitrant seeds & pollen: tissue culture supplies, laminar flow hood, autoclave, dishwasher, media, pH meter, Petri dishes, labels, growth room, necessary solutions, culture vessels, plastic sealing film, compound microscope to measure pollen tube growth. For dormant buds: sterile peat moss for rehydration, rootstocks, grafting tape/rubber bands/knife, labels, greenhouse/growth chamber.
Documentation
Suitable designed database/genebank information management system aligned to FAO/Bioversity Multi-Crop Passport Descriptors and other data standards, e.g. GRIN-Global. Database with built-in automated tools for checking inventory and viability, ability to add/subtract inventories as needed, and flagging accessions requiring regeneration/replacement. Data backup/storage
Distribution
Balances, seed counter, envelopes bag sealer, labels, packing materials. Depending on propagule/distribution method: Cryoshipper, test tubes in a rack (for *in vitro* materials), packing boxes/envelopes, packing slip. Data sheets or mobile devices for electronic data recording, barcode reader, recipient documentation, permits and phytosanitary certificates.
Security and personnel
Generator(s), fire-extinguishing equipment, security cameras, alarm systems, security doors. Protective clothing and protective gear such as face masks, lab coats, gloves and footwear.

13. References cited

Acker, J.P., Adkins, S., Alves, A., Horna, D. & Toll, J. 2017. *Feasibility study for a safety back-up cryopreservation facility*. Independent expert report: July 2017. Rome (Italy), Bioversity International, 100 pp.

Alercia, A., Diulgheroff, S. & Mackay, M. 2015. *FAO/Bioversity multi-crop passport descriptors V.2.1*. https://api.semanticscholar.org/CorpusID:135350643

Amphasys AG. 2022. *Pollen Bioprocessing***.** Lucerne, Switzerland. Cited 29 October 2024. https://amphasys.com/

Araújo de Oliveira, A.C., da Silva Lédo, A., Polek, M., Krueger, R., Shepherd, A. & Volk, G.M. 2021. Optimization of in vitro germination and cryopreservation conditions for preserving date palm pollen in the USDA National Plant Germplasm System. *Plant Cell, Tissue and Organ Culture*), 144:223–232. https://doi.org/10.1007/s11240-020-01907-1

AVRDC (World Vegetable Center). 2012. *Material Transfer Agreement for Germplasm Accessions*. Shanhua, Taiwan Province of China. https://avrdc.org/?wpfb_dl=524

Ballesteros, D., Fanega-Sleziak, N. & Davies, R.M. 2021. Cryopreservation of seeds and seed embryos in orthodox-, intermediate-, and recalcitrant-seeded species. In: Wolkers, W.F., Oldenhof, H. (eds) Cryopreservation and freeze-drying protocols. *Methods in Molecular Biology*, vol 2180. Humana, New York, NY. https://doi.org/10.1007/978-1-0716-0783-1_36

Ballesteros, D. & Pritchard, H.W. 2020. The cryobiotechnology of oaks: An integration of approaches for the long-term *ex situ* conservation of Quercus species. *Forests*, 11: e1281. https://doi.org/10.3390/f11121281

Benson, E.E., Harding, K., Debouck, D., Dumet, D., Escobar F., Mafla, G., Panis, B., Panta, A., Tay, D., Van den houwe, I. & Roux, N. 2011a. *Refinement and standardization of storage procedures for clonal crops - Global Public Goods Phase 2*: Part I. Project landscape and general status of clonal crop in vitro conservation technologies. System-wide Genetic Resources Programme, Rome, Italy.

Benson, E.E., Harding, K., Debouck, D., Dumet, D., Escobar, R., Mafla, G., Panis, B., Panta, A., Tay, D., Van den houwe, I., Roux, N. 2011b. *Refinement and standardization of storage procedures for clonal crops - Global Public Goods Phase 2*: Part III. Multi-crop guidelines for developing in vitro conservation best practices for clonal crops. System-wide Genetic Resources Programme, Rome, Italy.

Bettoni, J.C., Bonnart, R., Shepherd, A., Kretzschmar, A.A. & Volk, G.M. 2019a. Successful cryopreservation of *Vitis vinifera* 'Chardonnay' from both *in vitro* and growth chamber source plants. *Acta Horticulturae*, 1234:211–217. https://doi.org/10.17660/A*ctaHortic*.2019.1234.28

Bettoni, J.C., Bonnart, R., Shepherd, A., Kretzschmar, A.A., Volk, G.M. 2019b. Cryopreservation of grapevine (*Vitis* spp.) shoot tips from growth chamber-sourced plants and histological observations. VITIS-*Journal of Grapevine Research*, 58:71–78. https://doi.org/10.5073/vitis.2019.58.71-78

Bettoni, J.C., Bonnart, R. & Volk, G.M. 2021. Challenges in implementing plant shoot tip cryopreservation technologies. *Plant Cell, Tissue and Organ Culture*, 144:21–34. https://doi.org/10.1007/s11240-020-01846-x

Bettoni, J.C., Marković, Z., Bi, W., Volk, G.M., Matsumoto, T. & Wang, Q.-C. 2021. Grapevine Shoot Tip Cryopreservation and Cryotherapy: Secure Storage of Disease-Free Plants. *Plants*, 10:2190. https://doi.org/10.3390/plants101021 90

Bettoni, J.C., Chen, K. & Volk, G.M. 2024. An Overview of Shoot Tip Cryopreservation. In: Volk GM (Eds.) *Training in Plant Genetic Resources: Cryopreservation of Clonal Propagules*. Fort Collins, Colorado: Colorado State University. 20 October 2024. https://colostate.pressbooks.pub/clonalcryopreservation/chapter/an-overview-of-shoot-tip-cryopreservation/

Bettoni, J.C. & Volk, G.M. 2024. Cryotherapy Using Shoot Tip Cryopreservation. In: Volk GM (Eds.) *Training in Plant Genetic Resources: Cryopreservation of Clonal Propagules*. Fort Collins, Colorado: Colorado State University. 21 October 2024. https://colostate.pressbooks.pub/clonalcryopreservation/chapter/cryotherapy-using-shoot-tip-cryopreservation/

Bettoni, J.C., Wang, M-R., Li, J-W., Fan, Xudong, Fazio, G., Hurtado-Gonzales, O., Volk, G.M. & Wang, Q-C. 2024. Application of biotechniques for in vitro virus and viroid elimination in pome fruit crops. *Phytopathology*, 114:930-954. https://doi.org/10.1094/PHYTO-07-23-0232-KC

CBD (Convention on Biological Diversity). 2018. *Frequently asked questions on access and benefit-sharing (ABS)*. Montreal, Canada. https://www.cbd.int/abs/doc/abs-factsheet-faq-en.pdf

CGIAR Genebank Platform. 2021. Quality Management. Bonn, Germany. Cited 29 October 2021. https://www.genebanks.org/the-platform/quality-management/

Dussert, S., Chabrillange, N., Engelmann, F., Anthony, F., Louarn, J., & Hamon, S. 1998. Cryopreservation of seeds of four coffee species (*Coffea arabica*, *C. costatifructa*, *C. racemosa* and *C. sessiliflora*): importance of water content and cooling rate. *Seed Science Research* 8(1):9-15. doi:10.1017/S096025850000386X

Dussert, S., Engelmann, F., & Noirot, M. 2003. Development of probabilistic tools to assist in the establishment and management of cryopreserved plant germplasm collection. *CryoLetters* 24(3):149-160.

Ellis, D., Skogerboe, D., Andre, C., Hellier, B. & Volk, G. 2006. *Implementation* of garlic cryopreservation techniques in the National Plant Germplasm System. *CryoLetters*, 27:99–106. PMID: 16794741.

Engelmann, F. 2004. Plant cryopreservation: Progress and prospects. *In Vitro Cell.Dev.Biol.-Plant* 40, 427–433. https://doi.org/10.1079/IVP2004541

FAO (Food and Agriculture Organization of the United Nations). 2021a. *Digital Object Identifiers (DOI)*. Rome. Cited 29 October 2024. http://www.fao.org/plant-treaty/areas-of-work/global-information-system/doi/en

FAO. 2021b. *The Multilateral System*. Rome. Cited 29 October 2024. https://www.fao.org/plant-treaty/areas-of-work/the-multilateral-system/the-smta/en

FAO. *2021c.* *Easy-SMTA Homepage*. Rome. Cited 29 October 2024. https://mls.planttreaty.org/itt/

FAO. 2014. *Genebank Standards for Plant Genetic Resources for Food and Agriculture*. Rev. ed. Rome.

FAO. 2022a. *Practical guide for the application of the Genebank Standards for Plant Genetic Resources for Food and Agriculture: Conservation of orthodox seeds in seed genebanks*. Commission on Genetic Resources for Food and Agriculture. Rome. https://doi.org/10.4060/cc0021en

FAO. 2022b. *Practical guide for the application of the Genebank Standards for Plant Genetic Resources for Food and Agriculture: Conservation in field genebanks*. Commission on Genetic Resources for Food and Agriculture. Rome. https://doi.org/10.4060/cc0023en

FAO. 2022c. *Practical guide for the application of the Genebank Standards for Plant Genetic Resources for Food and Agriculture: Conservation via* in vitro *culture*. Commission on Genetic Resources for Food and Agriculture. Rome. https://doi.org/10.4060/cc0025en

FAO. 2025. *Practical guide for the application of the Genebank Standards for Plant Genetic Resources for Food and Agriculture: Conservation in genebanks of species producing non-orthodox seeds*. Commission on Genetic Resources for Food and Agriculture. Rome.

Global Crop Diversity Trust. 2024. *GGCE Software for crop genebank*. Bonn, Germany. Cited 7 October 2025. https://ggce.genesys-pgr.org/

Gold, K. & Hay, F. 2014. *Technical Information Sheet_09: Equilibrating seeds to specific moisture levels*. Board of Trustees of the Royal Botanic Gardens, Kew. http://brahmsonline.kew.org/Content/Projects/msbp/resources/Training/09-Seed-air-moisture-relations.pdf

GRIN-Global. 2021. *The GRIN Global Project*. Fort Collins, USA. Cited 7 October 2024. https://www.grin-global.org/

Guarino, L.G., Rao, L.R. & Reid, V., eds. 1995. *Collecting plant genetic diversity*. Technical guidelines. Wallingford, UK, CAB International. http://www.bioversityinternational.org/elibrary/publications/detail/collecting-plant-genetic-diversity/

Harding, K. & Benson, E. 2015. Writing standard operating procedures (SOPs) for cryostorage protocols: Using shoot meristem cryopreservation as an example. In Willem F. Wolkers and Harriëtte Oldenhof (eds.), *Cryopreservation and freeze-drying protocols, Methods in Molecular Biology, Springer Protocols,* New York. vol. 1257, DOI 10.1007/978-1-4939-2193-5_22

Höfer, M. 2015. Cryopreservation of winter-dormant apple buds: establishment of a duplicate collection of *Malus* germplasm. *Plant Cell Tissue Organ Culture* 121, 647–656. https://doi.org/10.1007/s11240-015-0735-1

Hor,Y.L., Chin,H.F. & Karim,M.Z. 1984. The effect of seed moisture and storage temperature on the storability of cocoa (*Theobroma cacao*) seeds. *Seed Science and Technology* 12:415-420,

IITA (International Institute of Tropical Agriculture). 2012. *Standard Operation Procedures (SOP) for IITA Seedbank*. Ibadan, Nigeria. https://www.iita.org/wp-content/uploads/2017/SOP_for_IITA_Seedbank.pdf

ILO (International Labour Organization). 2021. *Country profiles on occupational safety and health and labour inspection*. Geneva, Switzerland. Cited 9 Nov 2024. https://www.ilo.org/global/topics/safety-and-health-at-work/country-profiles/lang--en/index.htm

International Potato Center. 2023.*Cryopreservation of potato shoot tips*. https://www.youtube.com/watch?v=7y-qWW08B5Y Accessed on: Oct 23, 2024

IPPC (International Plant Protection Convention). 2021. *List of NPPOs of IPPC Contracting parties*. Rome. Cited 29 October 2021. https://www.ippc.int/en/countries/nppos/listcountries/

ISBER. 2023. *Best Practices: Recommendations for repositories, 5th Edition*. 196 pg. https://www.isber.org/page/BP5Downloaded

Jenderek, M., Ambruzs, B., Holman, G., Castens, J., Ellis, D. & Widrlechner, M. 2020. Salix dormant buds cryotolerance varies by taxon, harvest year, and stem-segment length. *Crop Science* 60:1965–1973. https://doi.org/10.1002/csc2.20135

Kim, H.-H. & Popova, E. 2023.Unifying principles of cryopreservation protocols for new plant materials based on alternative cryoprotective agents (CPAs) and a systematic approach. *CryoLetters*, 44:1–12. https://doi.org/10.54680/fr23110110112

Lei, X., Wang, Q., Yang, H., Qi, Y., Hao, X. & Wang, Y. 2021. Vitrification and proteomic analysis of embryogenic callus of *Panax ginseng* C.A. Meyer. *In Vitro Cellular & Developmental Biology-Plant*, 57:118-127. https://doi.org/10.1007/s11627-020-10117-5

Nagel, M., Pence, V., Ballesteros, D., Lambardi, M., Popova, E. & Panis, B. 2024. Plant cryopreservation: Principles, applications, and challenges of banking plant diversity at ultralow temperatures. *Annual Review of Plant Biology*, 75:797–824. https://doi.org/10.1146/annurev-arplant-070623-103551

Nadarajan, J., Walters, C., Pritchard, H.W., Ballesteros, D. & Colville L. 2023. Seed Longevity—The Evolution of Knowledge and a Conceptual Framework. *Plants*,12:471. https://doi.org/10.3390/plants12030471

Nebot, A., Philpott, V.J., Pajdo, A., Ballesteros, D. 2021. Cryopreservation of fern spores and pollen. In: Wolkers, W.F., Oldenhof, H. (eds). *Cryopreservation and Freeze-Drying Protocols. Methods in Molecular Biology, vol 2180*. Humana, New York, NY. https://doi.org/10.1007/978-1-0716-0783-1_33

Niino, T., Yamamoto, S., Matsumoto, T., Elgelmann, F., Valle Arizaga, M. & Tanaka, D. 2019. Development of V and D cryo-plate methods as effective protocols for cryobanking. *Acta Horticulturae*, 1234:249–262. https://doi.org/10.17660/ActaHortic.2019.1234.33

Nishizawa, S., Sakai, A., Amano, Y. & Matsuzawa, T. 1993. Cryopreservation of asparagus (*Asparagus officinalis* L.) embryogenic suspension cells and subsequent plant regeneration by vitrification. *Plant Science*, 91:67–73. https://doi.org/10.1016/0168-9452(93)90189-7

Nits, O.S., Sementsova, M.V., Osipova, E.S., Tereshonok, D.V. & Gladkov EA. 2024. IPPRAS Cryobank for the Conservation of Orthodox Seeds of Rare, Endangered, Medicinal, and Ornamental Plant Species—Current Research. *Plants*, 13:1354. https://doi.org/10.3390/plants13101354

Panis B. 2009. *Cryopreservation of* Musa *germplasm: 2nd edition. Technical Guidelines No. 9* (F. Engelmann and E. Benson, eds). Bioversity International, Montpellier, France.

Panis, B., Nagel, M. & Van den houwe, I. 2020. Challenges and prospects for the conservation of crop genetic resources in field genebanks, in *in vitro* collections and/or in liquid nitrogen. *Plants*, 9:1634. https://doi.org/10.3390/plants9121634

Powerpollen. 2024. *Pollination on demand*. Ames, ISA. Cited 29 October 2024. https://powerpollen.com/

Pritchard, H.W., & Nadarajan, J. 2008. Cryopreservation of orthodox (desiccation tolerant) seeds. In *Plant cryopreservation: a practical guide* (pp. 485–501). New York, NY: Springer New York.

Rajasekharan, P.E. & Rohini, M.R., eds. 2023. *Pollen Cryopreservation Protocols*. Springer Nature.

Reed, B.M. ed., 2008. *Plant cryopreservation: a practical guide* (p. 513). New York: Springer.

Ren, L., Wang, M.R. & Wang, Q.C. 2021. ROS-induced oxidative stress in plant cryopreservation: occurrence and alleviation. *Planta* 254, 124.

Roach, T., Ivanova, M., Beckett, R.P., Minibayeva, F.V., Pritchard, H.W. & Kranner, I. 2008. An oxidative burst of superoxide in embryonic axes of recalcitrant sweet chestnut seeds as induced by excision and desiccation. *Physiologia Plantarum*, 133:131–139. https://doi.org/10.1111/j.1399-3054.2007.00986.x

SGRP-CGIAR. 2010. *Crop Genebank Knowledge Base - Guidelines for testing germination of the most common crop species*. Rome. Cited 9 Nov. 2024. https://cropgenebank.sgrp.cgiar.org/images/file/procedures/guidelines%20for%20testing%20germination%20of%20the%20most%20common%20crop%20species.pdf

Sakai, A., Kobayashi, S. & Oiyama, I. 1990. Cryopreservation of nucellar cells of navel orange (*Citrus sinensis* Osb. var. *brasiliensis* Tanaka) by vitrification. *Plant Cell Reports*, 9:30–33. https://doi.org/10.1007/BF00232130

Tanner, J.D., Chen, K.Y., Bonnart, R.M., Minas, I.S. & Volk, G.M. 2021a. Considerations for large-scale implementation of dormant budwood cryopreservation. *Plant Cell, Tissue and Organ Culture*, 144:35–48. https://doi.org/10.1007/s11240-020-01884-5

Tanner, J.D., Chen, K.Y., Jenderek, M.M, Wallner, S.J. & Minas, I.S. 2021b. Determining the effect of pretreatments on freeze resistance and survival of cryopreserved temperate fruit tree dormant buds. *Cryobiology*, 101:87–94. https://doi.org/10.1016/j.cryobiol.2021.05.003

Tanner, J.D., Minas, I.S., Chen, K.Y., Jenderek, M.M. & Wallner, S.J. 2020. Antimicrobial forcing solution improves recovery of cryopreserved temperate fruit tree dormant buds. *Cryobiology*, 92:241–247. https://doi.org/10.1016/j.cryobiol.2020.01.019

Uchendu, E.E., Leonard, S.W., Traber, M.G. & Reed, B.M. 2010a. Vitamins C and E improve regrowth and reduce lipid peroxidation of blackberry shoot tips following cryopreservation. *Plant Cell Reports*, 29:25–35. https://doi.org/10.1007/s00299-009-0795-y

Uhendu, E.E., Muminova, M., Gupta, S. & Reed, B.M. 2010b. Antioxidant and anti-stress compounds improve regrowth of cryopreserves Rubus shoot tips. *In Vitro Cellular & Developmental Biology – Plant*, 46:386–393. https://doi.org/10.1007/s11627-010-9292-9

Volk, G. (ed.). 2020. T*raining in plant genetic resources: Cryopreservation of clonal propagules*. US Department of Agriculture. Cited 7 October 2024: https://colostate.pressbooks.pub/clonalcryopreservation/

Volk, G.M., Bonnart, R., Araújo de Oliveira, A.C. & Henk, A.D. 2022. Minimizing the deleterious effects of endophytes in plant shoot tip cryopreservation. *Applications in Plant Science*, 10:e11489. https://doi.org/10.1002/aps3.11489

Volk, G.M., Bonnart, R. & Shepherd, A. 2020. Citrus Micrografting for Regrowth After Shoot Tip Cryopreservation. In: Volk GM (Eds.) *Training in Plant Genetic Resources: Cryopreservation of Clonal Propagules*. Fort Collins, Colorado: Colorado State University. 11 August 2024. https://colostate.pressbooks.pub/clonalcryopreservation/chapter/citrus-micrografting/

Volk, G.M., Bonnart, R., Shepherd, A., Yin, Z., Lee, R., Polek, M.L. & Krueger, R. 2015. Citrus cryopreservation: viability of diverse taxa and histological observations. *Plant Cell, Tissue and Organ Culture*, 128:327–334. https://doi.org/10.1007/s11240-016-1112-4

Volk, G.M., Crane, J., Caspersen, A.M., Kovach, D., Gardner, C. & Walters, C. 2007. Hydration of *Cuphea* seeds containing crystallized triacylglycerols. *Functional Plant Biology*, 34:360–367. https://doi.org/ 10.1071/FP06291

Volk, G.M., Henk, A.D., Jenderek, M.M. & Richards, C.M. 2017. Probabilistic viability calculations for cryopreserving vegetatively propagated collections in genebanks. *Genetic Resources and Crop Evolution* 64:1613–1622. https://doi.org/10.1007/s10722-016-0460-6

Volk, G.M., Jenderek, M. & Chen, K. 2020b. Cryopreservation of Dormant Apple Buds. In: Volk GM (Eds.) *Training in Plant Genetic Resources: Cryopreservation of Clonal Propagules*. Fort Collins, Colorado: Colorado State University. 11 August 2024. https://colostate.pressbooks.pub/clonalcryopreservation/chapter/apple-dormant-bud-cryopreservation/

Volk, G.M., Krueger, R., Moreland, B., Bonnart, R. & Shepherd A. 2020. Citrus Shoot Tip Cryopreservation. In: Volk GM (Eds.) *Training in Plant Genetic Resources: Cryopreservation of Clonal Propagules*. Fort Collins, Colorado: Colorado State University. Date accessed. Available from https://colostate.pressbooks.pub/clonalcryopreservation/chapter/citrus-cryopreservation/

Vollmer, R., Villagaray, R., Egusquiza, V., Espirilla, J., Garcia, M., Torres, A., Rojas, E., Panta, A., Barkley, N. A., Ellis, D. 2016. The potato cryobank at the International Potato Center (CIP): a model for long term conservation of clonal plant genetic resources collections of the future. *CryoLetters*. (UK). ISSN 0143-2044. 37(5):318-329.

Vollmer R, Villagaray R, Cárdenas J, Castro M, Chávez O, Anglin NL, Ellis D. A large-scale viability assessment of the potato cryobank at the International Potato Center (CIP). *In Vitro Cell Dev Biol Plant.* 2017;53(4):309-317. doi: 10.1007/s11627-017-9846-1.

Vollmer, R., Villagaray, R., Cárdenas, J., Castro, M., Chávez, O., Anglin, N.L. & Ellis, D. 2017. A large-scale viability assessment of the potato cryobank at the International Potato Center (CIP*). In Vitro Cellular & Developmental Biology – Plant*, ;53:309–317. https://doi.org/10.1007/s11627-017-9846-1.

Vollmer, R., Espirilla, J., Villagaray, R., Cárdenas, J., Castro, M., Sánchez, J.C., Manrique-Carpintero, N., Ellis, D., Anglin N.L. 2021**.** Correction to: Cryopreservation of Potato Shoot Tips for Long-Term Storage. In: Dobnik, D., Gruden, K., Ramšak, Ž., Coll, A. (eds) Solanum tuberosum. *Methods in Molecular Biology*, vol 2354. Humana, New York, NY. https://doi.org/10.1007/978-1-0716-1609-3_21

Vollmer, R., Villagaray, R., Cárdenas, J., Castro, M., Chávez, O., Anglin, N.L. & Ellis, D. 2017. A large-scale viability assessment of the potato cryobank at the International Potato Center (CIP*). In Vitro Cellular & Developmental Biology – Plant*, ;53:309–317. https://doi.org/10.1007/s11627-017-9846-1.

Vollmer, R., Villagaray, R., Castro, M., Anglin, N.L. & Ellis, D. 2019. Cryopreserved potato shoot tips showed genotype-specific response to sucrose concentration in rewarming solution (RS). *Plant Cell, Tissue and Organ Culture*, 136, 353–363 https://doi.org/10.1007/s11240-018-1520-8

Vollmer, R., Villagaray, R., Castro, M., Cárdenas, J., Pineda, S., Espirilla, J., Anglin, N., Ellis, D. & Rennó Azevedo, V.C. 2022. The world's largest potato cryobank at the International Potato Center (CIP) - Status quo, protocol improvement through large-scale experiments and long-term viability monitoring. *Frontiers in Plant* Science, 13:1059817. https://doi.org/10.3389/fpls.2022.1059817.

Walters, C., Volk, G.M. & Richards, C.M. 2008. Genebanks in the post-genomic age: Emerging roles and anticipated uses. *Biodiversity*, 9:68–71. https://doi.org/10.1080/14888386.2008.9712887

Walters, C., Wesley-Smith, J., Crane, C., Hill, L.M., Chmielarz, P., Pammenter, N.W., & Berjak, P. 2008. Cryopreservation of recalcitrant (i.e. desiccation-sensitive) seeds. In: B.M Reed (ed.) *Plant cryopreservation: a practical guide*. Springer.

Walters, C., Wheeler, L. & Stanwood, P.C. 2004. Longevity of cryogenically stored seeds. *Cryobiology*, 48:229–244. https://doi.org/10.1016/j.cryobiol.2004.01.007

Wang, M.R., Bi, W-L., Bettoni, J.C., Zhang, D., Volk, G.M., Wang, Q-C. 2022. Shoot tip cryotherapy for plant pathogen eradication. *Plant Pathology*, 71:1241–1254. https://doi.org/10.1111/ppa.13565

Wang, M.R., Bi, W-L., Shukla, M.R., Ren, L, Hamborg, Z., Blystad, D-R., Saxena, P.K. & Wang, Q-C. 2021. Epigenetic and Genetic Integrity, Metabolic Stability, and Field Performance of Cryopreserved Plants. *Plants*, 10:1889. https://doi.org/10.3390/plants10091889

Zhang, A.-L., Wang, M.-R., Li, Z., Panis, B., Bettoni, J.C., Vollmer, R., Xu, L. & Wang, Q.-C. 2023. Overcoming Challenges for Shoot Tip Cryopreservation of Root and Tuber Crops. *Agronomy*, 13:219. https://doi.org/10.3390/agronomy 13010219

Zhao, L., Wang, M., Li, J., Cui, Z., Volk, G.M. & Wang, Q. 2019. Cryobiotechnology: A double-edged sword for obligate plant pathogens. *Plant Disease*, 103:1058–1067. https://doi.org/10.1007/s10722-016-0460-6

14. Further information / reading

Center for Plant Conservation. 2018. *CPC best plant conservation practices to support species survival in the wild*. Center for Plant Conservation.

Gonzalez-Arnao, M.T., Martinez-Montero, M.E., Cruz-Cruz, C.A. & Engelmann, F. 2014. Advances in cryogenic techniques for the long-term preservation of plant biodiversity. *Biotechnology and Biodiversity*, pp.129–170. https://doi.org/10.1007/978-3-319-09381-9_8

Keller, E.R., Kaczmarczyk, A. & Senula. A. 2008. Cryopreservation for plant genebanks-a matter between high expectations and cautious reservation. *CryoLetters*, 29:53–62. PMID: 18392290

Li, D.Z. & Pritchard, H.W. 2009. The science and economics of *ex situ* plant conservation. *Trends in Plant Science*, *14*:614–621. https://doi.org/10.1016/j.tplants.2009.09.005

Ougham, H. & Thomas, I.D. 2013. Germplasm databases and informatics. In: M. Jackson, B., Ford-Lloyd & M. Parry, eds. *Plant genetic resources and climate change*, pp.151–165. Wallingford, UK, CAB International.

Painting, K.A, Perry, M.C, Denning, R.A. & Ayad, W.G. 1993. *Guidebook for genetic resources documentation*. Rome, IPGRI. https://www.bioversityinternational.org/fileadmin/_migrated/uploads/tx_news/Guidebook_for_genetic_resources_documentation_432.pdf

Pritchard, H.W. 2016. Priority science for the preservation of priority crops. *Indian Journal of Plant Genetic Resources*, 29:292–297. https://doi.org/10.5958/0976-1926.2016.00049.8

Walters, C. and Pence, V.C. 2021. The unique role of seed banking and cryobiotechnologies in plant conservation. *Plants, People, Planet*, 3:83–91. https://doi.org/10.1002/ppp3.10121

Wang, M.R., Lambardi, M., Engelmann, F., Pathirana, R., Panis, B., Volk, G.M. & Wang, Q.C. 2021. Advances in cryopreservation of in vitro-derived propagules: Technologies and explant sources. *Plant Cell, Tissue and Organ Culture*, 144:7–20. https://doi.org/10.1007/s11240-020-01770-0

Wolkers, W.F. & Oldenhof, H. (eds.). 2022. *Cryopreservation and Freeze-Drying Protocols, Fourth Edition*. Methods in Molecular Biology, Springer Protocols, Humana Press, 742 pp.

Annex: Risks and Associated Mitigation

It is important that staff are properly trained and follow documented procedures at all stages of genebank operations. Specific risks to be considered during genebank operations are presented below.

Acquisition

Risk	Risk control/mitigation
Diversity of the source population is not adequately represented in the preserved sample	▪ Develop and follow an agreed cryopreservation strategy and methodology that adequately follow genetic sampling guidelines.
Mislabeling/loss of labels	▪ Use appropriate labels when samples are collected and transported.
Transcription errors	▪ Consider the use of barcodes, ensuring regular data backup and availability of sufficient charged batteries. ▪ Implement data validation.
Loss of viability during transport to the cryobank	▪ Ensure timely transfer to controlled conditions. ▪ Ensure appropriate post-harvest handling according to propagule maturity, prevailing environmental conditions and phytosanitary conditions.

Cryopreservation of diverse propagule types

Risk	Risk control/mitigation
Reduced propagule longevity	▪ Ensure appropriate propagule preparation and cryostorage conditions, optimizing as necessary. ▪ Document methods for cryopreservation and viability/regrowth so others can repeat the process (especially the regrowth process).
Mixing/mislabeling of samples	▪ Label carefully to avoid mixing. ▪ Use computer-generated barcode labels to minimize errors.
Stored sample falls below viability or quantity thresholds	▪ Ensure that the documentation system includes automated tools to monitor viability and inventory and flag up accessions requiring regeneration. ▪ Install a monitoring system for tanks to ensure temperature is maintained and monitor LN tank levels routinely. ▪ Ensure sufficient supply of LN for replacement purposes. ▪ Separate packets/vials aliquoted for regrowth/regeneration/distribution without affecting the other samples. ▪ Conduct only planned rewarming activities.

Viability/regrowth assessment, monitoring of cryopreserved propagules and post-cryopreservation quality assessment

Risk	Risk control/mitigation
Poorly recorded, unreliable data	▪ Well-trained staff. ▪ Mobile devices to record data. ▪ Record all necessary information in the information management system. ▪ Data validation by curator and/or documentation officer.
Misidentification of sample	▪ Check container labels while collecting data.
Reduced propagule longevity	▪ Ensure that samples are not inadvertently warmed while retrieving from tank.

Replacement of cryopreserved inventories

Risk	Risk control/mitigation
Poorly recorded, unreliable data	▪ Well-trained staff. ▪ Mobile devices to record data. ▪ Record all necessary information in the information management system. ▪ Record detailed cryopreservation and regrowth/regeneration methods. ▪ Data validation by curator and/or documentation officer.
Materials are not true-to-type	▪ Coordinate among curation teams to send regenerated plants to be evaluated for phenotypes and genotypes.
Misidentification of sample/accession	▪ Check container and pot labels; use bar codes.

Distribution and safety duplication

Risk	Risk control/mitigation
Mixing/mislabelling of samples	▪ Careful packaging to avoid mixing. ▪ Labels placed inside and outside of package. ▪ Use computer-generated barcode labels to minimize errors.
Viability loss due to delayed or damaged shipments	▪ Ensure shipping documents are accurate and available: import permit, phytosanitary permit, agreements, etc. ▪ Ensure samples are dispatched promptly and use the fastest and safest way of sending. ▪ Ensure recipient is expecting materials and knows how to receive/process/store.