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COMMISSION ON GENETIC RESOURCES FOR FOOD AND AGRICULTURE

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DRAFT 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

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1. Introduction

The majority of plant species, including many of the most important food crops, produce orthodox seeds that can be dried to a low moisture content and stored at low temperatures. Lowering seed moisture content and storage temperature extends the storage life of orthodox seeds, with seeds of some species potentially maintaining high levels of viability for tens or even hundreds of years. Species that produce orthodox seeds, and which can therefore be conserved in conventional seed genebanks, include cereals, grain legumes, forages, most vegetables and some fruits. Most wild relatives of these crops also produce orthodox seeds. Some crops that are usually propagated vegetatively, for example potato, also produce true seeds that are orthodox.

A small proportion of species produce seeds that show significantly reduced or no germination following conventional seed genebank storage, though the point or time in the conservation cycle when this becomes apparent varies. Recalcitrant seeds do not tolerate removal of water that will freeze upon exposure to sub-zero temperatures and, if they are frozen at high moisture content, formation of ice crystals damages the tissues irrevocably. Depending on how much moisture loss can be survived, they may be further classified as extremely or minimally recalcitrant, with the latter tolerating more moisture loss than the former.

This variability in degree of desiccation sensitivity can be extended: seed storage behaviour is often described as a continuum between extremely short-lived, desiccation-sensitive recalcitrant seeds and extremely long-lived orthodox seeds. Somewhere along this continuum, there are seeds which are described as having a storage response that is 'intermediate' to recalcitrant or orthodox seed storage behaviour. Such seeds commonly survive drying at 50-65% RH or lower, but may

(i) be damaged if dried below 25% RH;

(ii) lose viability faster in the freezer (-18°C) than in the cold store (4-8°C).

Because intermediate seeds have to be stored at a higher moisture content and/or temperature than used for orthodox seeds, they generally have shorter storage periods than many orthodox species in conventional genebank storage.

While seed storage behaviour is not documented for all species, it has been predicted that overall, 92% of angiosperm species will produce orthodox seeds. It is also recognized that recalcitrant seed storage behaviour has only been documented within certain plant families (Wyse and Dickie, 2017). However, in general, if someone has decided that seeds of a particular species should be deposited in a genebank, the seed storage behaviour will either be known and documented, or it is likely that there is some accompanying information which indicates the likely seed storage behaviour. Some examples of economically important species which produce non-orthodox seeds are provided in the Appendix. If a genebank does receive or collect seeds of species for which seed storage behaviour has not been documented and non-orthodox behaviour is suspected, the genebank is encouraged to do research to characterize the seed storage behaviour[1], including, for intermediate non-orthodox seeds in particular, the optimum storage conditions.[2] In reality, most genebanks will not have access to the necessary equipment and supplies, or staff time available to conduct complex experiments. Indeed, it is important that the genebank consider the practicalities and priorities in relation to handling non-orthodox seeds. Above all, it should be emphasized that it is important to ensure that the material is in effective long-term conservation, which may not be as seeds and may be at another, partner genebank.

The conservation of species producing non-orthodox seeds can be broken down into a series of interrelated operations (Figure 1). This practical guide presents practices and activities critical to the underlying genebank principles in each operational area (Table 1). It outlines workflows for routine genebank operations for the handling of recalcitrant seeds (Figure 2) and supports the application of the *Genebank Standards for Plant Genetic Resources for Food and Agriculture* (Genebank Standards) (FAO, 2014). The purpose of this guide is to present the information contained in the Genebank Standards in a format that details the different actions of the genebank workflow in a sequential manner and thereby facilitate more widespread application of the Genebank Standards. Genebanks

[1] See section 5.
[2] See section 4.

may use the activities outlined in this guide as a basis for the development of standard operating procedures (SOPs) (e.g. IITA, 2012) and quality management systems (QMS) (CGIAR Genebank Platform, 2021) for conserving germplasm collections, defining in detail how to carry out each activity.

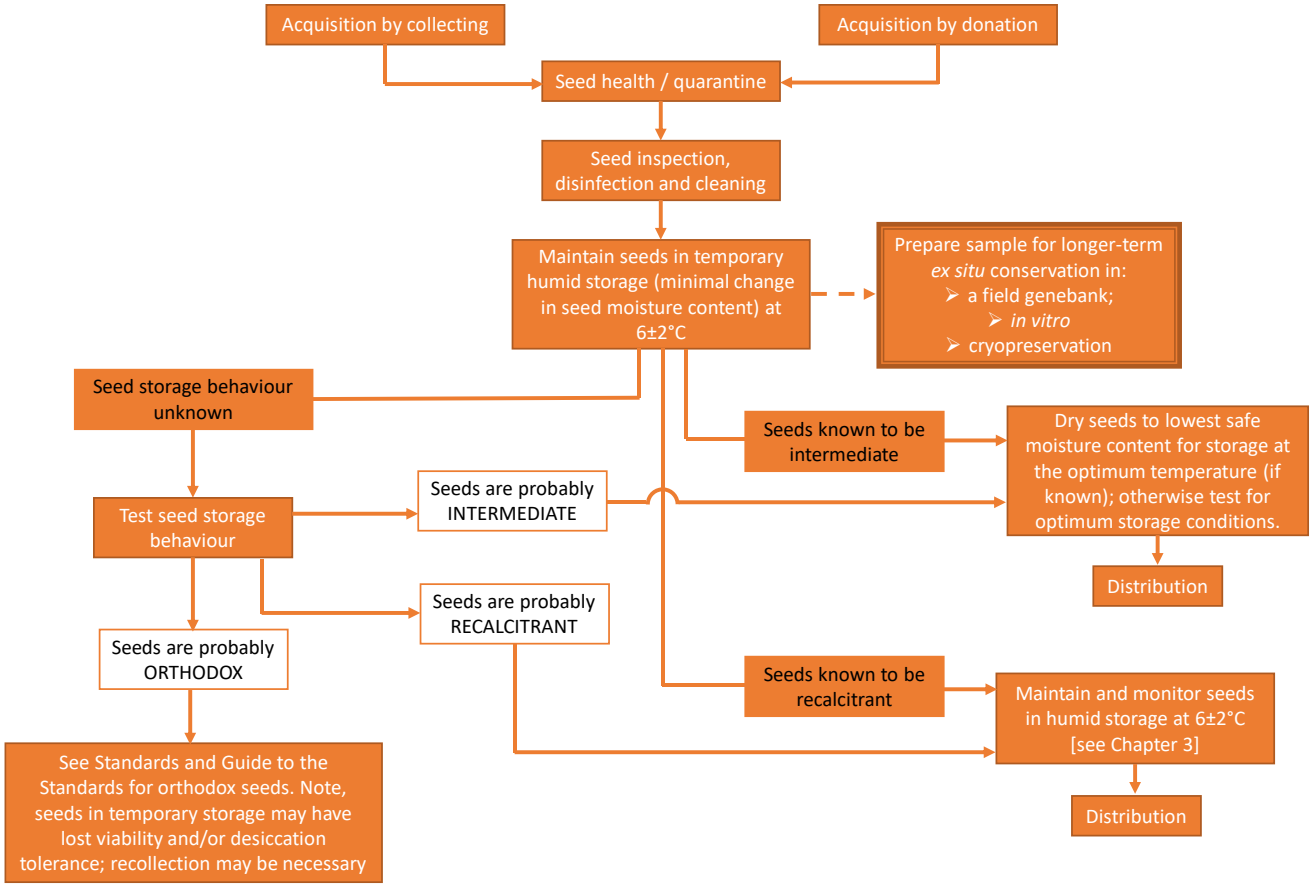
This booklet only provides general guidance on the complex steps and decisions required when conserving species producing non-orthodox seeds in a genebank. Each genebank will have its own unique and special circumstances, and the efficient management of particular collections will require careful consideration and procedural adjustments based on experience. For detailed technical specifications for 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.

Figure 1. Major operations for conservation of species producing non-orthodox seeds

Table 1. Underlying principles and related genebank operations for species producing non-orthodox seeds

Genebank principle	Summarized genebank operations
Identity of accessions	Passport data collected and recorded Taxonomic 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 genebank operations and in storage
Identity of inventory	Unique inventory number assigned to specific seed sample
Maintenance of viability	Best practices followed and optimized during collecting, temporary and short-term storage, seed testing, processing and transportation Storage conditions optimized and monitored
Maintenance of genetic integrity	Collection and maintenance of samples conducted in a manner that ensures they represent the original population as fully as possible Best practices followed during packing, regeneration and multiplication
Maintenance of germplasm health	Quarantine procedures undertaken when needed Best practices followed during collecting, testing and processing Contamination monitored and managed
Physical security of collections	Risk management strategy developed and implemented Appropriate genebank infrastructure in place and maintained Safety duplication through alternative conservation methods (field, *in vitro*, cryopreservation)
Availability and use of germplasm	Germplasm acquired and distributed according to legal and phytosanitary requirements 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 backups Passport and other relevant data available and accessible to external users, as far as possible
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

Figure 2. Flow of germplasm for species with recalcitrant, intermediate or unknown seed storage behaviour.



2. 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]

In the case of species known or suspected to produce non-orthodox seeds, it is important that the genebank considers whether it has the appropriate facilities and resources to handle the germplasm appropriately and promptly, before the acquisition process is initiated.

✓ **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.

✓ **Decisions regarding whether to proceed with acquisition and how to handle seeds are guided by the expectation of non-orthodox seed storage behaviour based on prior knowledge or experience.**

If the habitat/ecology of the species is known, as a rough guide:

- Recalcitrant seed storage behaviour is more likely to occur in species growing in moist habitats where seeds are exposed to high humidity during development, maturation and shedding, and where ambient temperature does not fall below 0°C, than in temperate or drier habitats; and
- Desiccation sensitive seeds are often dispersed with high moisture content and are non-dormant.

In addition:

- Search for the species – or genus/genera from the same family, if the species is not listed – in the Seed Information Database (SER, INSR and RBGK, 2024), which contains information on seed storage behaviour for thousands of species.
- Search the scientific literature, both for articles where seed storage behaviour is reported but also other articles. For example, studies related to germination may provide insights into storage behaviour.
- Seed storage behaviour might also be inferred from traditional knowledge; whether or not the seeds are routinely stored for long periods (many months) of time and under what conditions.

✓ **Germplasm added to the collection is legally acquired and accompanied by all relevant documentation.[4]**

The process of germplasm acquisition is governed by national and international regulations such as phytosanitary/quarantine laws and the International Treaty on Plant Genetic Resources for Food

[3] See Figure 3 at the end of this section for a summary diagram of the workflow and activities for acquisition of germplasm.
[4] Standard 6.1.1.

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 also 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 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.

✓ **Germplasm added to the genebank collection is accompanied by associated data, as outlined in the FAO/Bioversity Multi-Crop Passport Descriptors.**[5]

It is recommended that all samples, 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 with the single accession must be clear, for example, through the use of accession numbers and/or DOI.

✓ **All acquisition data, including associated metadata, are recorded, validated and uploaded to the genebank 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 and minimizes human error.

2.1 Germplasm acquired through collecting missions

✓ **A clear strategy for germplasm collecting missions is developed according to the institute's mandate.**

Setting collection priorities prior to any collection mission is essential. It is recommended that a collecting proposal be developed that clearly states the purpose of the collecting mission, the target location and the methodology. It may be appropriate and useful to:

- emphasize the importance of conducting inventories and gap analyses to prevent duplicates and of having a clear strategy for collecting missions that considers taxon distribution, national inventories and gap analyses;
- establish a collaboration with an institute or experts from the targeted area and abide by regulations for collecting in that area; and
- plan the mission well in advance in order to ensure best practices and compliance with regulations and requirements.

✓ **Collected germplasm is legally acquired and accompanied by all relevant documentation.**[6]

The process of germplasm acquisition is governed by national and international regulations. The following information could assist in ensuring compliance with these regulations:

[5] Standard 6.1.2.
[6] Standard 6.1.1.

- The genebank should communicate with the appropriate designated authorities when there are questions regarding germplasm acquisition.
    - For collecting missions in other countries, it may be necessary to contact the National Focal Points for the Treaty or other designated authorities for germplasm acquisition.
    - For collecting missions in the genebank's country, it may be necessary to contact the national competent authority in order to ensure understanding of and compliance with national and local regulations.
- Collecting permits from national, regional or local authorities, as appropriate, may be required for collecting crop wild relatives or semi-domesticated germplasm in natural populations[7] *in situ*.
- When collecting from farmers' fields/stores or community areas, including some natural habitats, prior informed consent (PIC) may be required and mutually agreed terms (MAT) (see CBD, 2018) determined, according to relevant national, regional or international laws and regulations.

✓ **The genebank abides by national, regional and international phytosanitary and any other import regulations and requirements from the relevant authorities.**[8]

When germplasm is moved there is a risk of accidentally introducing plant pests and diseases along with the host plant material. The following steps may help mitigate such risks while ensuring compliance with regulations and requirements:

- for materials collected in another country, obtaining a phytosanitary certificate from the provider country and an import permit from the relevant authorities in the genebank's country (see IPPC, 2021);
- passing collected samples through the relevant quarantine process before transferring them to the genebank, if required; and
- multiplying collected accessions with insufficient seed quantity in containment or in an isolated area, according to the advice of the national phytosanitary authority.

✓ **Collecting missions are scheduled at the optimum stage for harvesting fruits/seeds of the target species.**[9]

Genebank staff should consult sources of information such as herbaria and plant identification handbooks regarding the target species.

- Plant phenology, and variability in phenology depending on local environmental conditions, should be taken into account. It may be necessary to monitor populations to ensure plants are at the appropriate stage for collection of the target propagule(s).
    - Fruits and seeds should be collected at 'peak maturity', just before they disperse from the plant. Indicators of fruit maturity such as colour may be used to determine maturity.

✓ **Fruits/seeds are collected from visibly healthy plants and do not show signs of disease or insect pest infestation or other damage.**

- Individual plants should be checked before sampling.
- In order to prevent potential phytosanitary contamination, avoid, if possible, collecting dispersed seeds from the ground, soiled seeds or seeds infested with saprophytic or pathogenic fungi/bacteria or insects.

[7] A group of individual plants that share a geographic area or region and have common traits.
[8] Standard 6.1.1.
[9] Standard 6.1.3.

✓ **Fruits/seeds are collected from an appropriate number of individual plants while avoiding the depletion of the natural population targeted for collecting.**[10]

The breeding system of the target species may be taken into consideration in order to define the number of plants to sample within a population and the number of propagules to collect, depending on the type of material (see SGRP-CGIAR, 2011; Hoban and Schlarbaum, 2014; Kashimshetty, Pelikan and Rogstad, 2017).

- To attain reasonable representativeness, it is recommended to harvest fruits/seeds from at least 30 parents for cross-fertilizing species and 60 parents for autogamous species, if possible.
    - The sample size for collecting will usually be limited compared to orthodox seeds; nevertheless, all attempts should be made to maximize the genetic diversity of the collected fruits/seeds.
    - Consider keeping maternal lines separate, especially if it is not possible to collect from many parents (van der Merwe et al., 2023).
    - As a general rule, collecting more than 20 percent of the available seeds of a wild population should be avoided in order to leave sufficient seeds for natural population renewal (Way, 2003).

✓ **Different parts of the plant may be taken for taxonomic verification**

- A herbarium specimen can be used for later verification of the taxonomy of the collected fruits/seeds and as a future reference (see RGBK, 2022a).
    - Sample whole plants or individual stems, branches, leaves, roots, flowers and/or fruits/seeds from the same plants from which fruits/seeds were collected.
- A leaf sample maybe used for later molecular verification of taxonomy.
    - Place a small sample (5-10 mm-diameter discs) of a young leaf into a vial containing silica.

✓ ***In situ* processing is carried out to reduce the volume of material to transport and to ensure viable material reaches the genebank.**

In some cases, it will be advantageous – or even necessary – to do some processing of material in the field, before packing and transport.

- Extracting seeds from fruits will reduce the volume of material that needs to be transported, however it may increase the risk of damage during transportation. It should be done for seeds borne in fleshy fruits as the wet flesh might otherwise ferment or accelerate the deterioration of the seed(s) inside.

✓ **Appropriate containers are used depending on the type of material collected.**

The type and size of container into which samples are placed will depend on the material being collected and whether the same container will be used for transport (see below). Once placed in the container, material should be kept in the shade to avoid exposure to direct sunlight and high temperature.

- Fruits or seeds must not be allowed to dry and, since they are likely to be metabolically active, require oxygen. They can be placed inside loosely tied polythene bags, ensuring that there is plenty of air with the seeds.

✓ **Collected samples are labelled and are not mixed during handling.**

All samples should be clearly labelled. This may extend to labelling to the level of the individual from which the material was collected.

[10] Standard 6.1.3.

- Use indelible ink or computer-generated labels (preferably with barcodes), if possible, on the collection receptacle to label the sample.
- Placing labels both inside and outside a container is a good practice. Protecting inside labels from deterioration, for example by placing the label in a sealed plastic bag or using moisture resistant labels, is useful if the material is not dry. It is recommended to keep a journal with all collection numbers assigned to each sample and additional information, as required.

✓ **Collected germplasm is accompanied by the associated data outlined in the FAO/Bioversity Multi-Crop Passport Descriptors and is preferably accompanied by a herbarium voucher specimen.**

A standardized collecting form is helpful for collecting the associated data for each sample obtained. Each sample is assigned a collection number so that the samples can be linked to the recorded information. The following information may be gathered:

- Taxonomic identification at species and intraspecific levels, plant population type, habitat and ecology, soil conditions at the collecting site, GPS coordinates and photo images in order to provide curators and users of the germplasm with an understanding of its original context.
    - The species name and authority should be checked in Plants of the World Online,[11] World Flora Online[12] or with recognized taxonomic experts.
- Associated data for each sample obtained as detailed in the FAO/Bioversity Multi-Crop Passport Descriptors (Alercia, Diulgheroff and Mackay, 2015; see Box 1).
- Information on the origin of the germplasm, traditional knowledge, cultural practices, etc., if collecting from farmers' fields/stores.
- For any herbarium voucher specimen obtained as a reference from a population (for example wild species), it is important to use the same collection number as that of the collected sample and associate it with the accession number in the database.

Box 1. Minimum passport data

As a minimum, collecting forms should contain:	
• Collecting number	• Latitude of collecting site
• Collecting institute name/code	• Longitude of collecting site
• Taxon name, as detailed/specific as possible	• Elevation of collecting site
• Common crop name	• Date of collecting
• Location of collecting site	• Biological status (wild, weedy, landrace, etc.)

[11] See https://powo.science.kew.org/.
[12] See https://www.worldfloraonline.org/.

✓ **The period between collecting and transfer to the genebank is as short as possible to limit loss and deterioration of the material.**[13]

Given the relatively short shelf-life of these propagules, it is essential that the material reaches the genebank as soon as possible after harvest, within days rather than weeks. This should also be considered at the planning stage, including assessing the likelihood and risks of potential delays.

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

The time needed for document processing, shipment/transit time and conditions (temperatures and/or humidity) are generally taken into account in order to ensure that the material reaches the destination genebank in good condition. The following considerations could decrease the risk of germplasm loss after collecting missions:

Packaging

- In the case of intact fruits and seeds, precautions should be taken to avoid risks of fungal or insect attacks during shipment.
    - If a pest has been observed and correctly identified, it may be necessary to apply pesticide before packing. Avoid any unnecessary chemical treatment, as it may be harmful to the collected samples and to people. If treatments are applied, declare them on each package and in accompanying documentation.
    - Inflated, loosely tied polythene bags can be used to maintain a humid, aerated environment around the fruits/seeds. Bags may then be placed in a rigid container.
- For smaller seeds, use of rigid cushioned envelopes or insulated packaging should protect samples in vials from crushing by mechanical mail sorters and deterioration.

Transport

- For long transit times by road, periodic aeration of the fruits/seeds may be necessary.
- 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.

2.2 Germplasm acquired through transfer/donation

✓ **Donated germplasm is legally acquired and accompanied by all relevant documentation.**[14]

- If the donating institute is from a country that is a signatory to the Treaty and the donated germplasm includes crops or species listed under Annex 1 of the Treaty (FAO, 1995), it is necessary to use the Standard Material Transfer Agreement (SMTA) (FAO, 2021b, c).
- If the donating institute is from a country that is not a Contracting Party to the Treaty or if the germplasm is not covered under Annex 1, it is still good practice to use the SMTA. However, a Material Transfer Agreement (MTA) could be developed (e.g. AVRDC, 2012).
- For donations from institutions, plant breeders or other germplasm providers without an MTA, it may be useful for the genebank to have a donor agreement spelling out the conditions of germplasm transfer to the genebank.

[13] Standard 6.1.4.
[14] Standard 6.1.1.

✓ **Donated germplasm is accompanied by the associated data outlined in the FAO/Bioversity Multi-Crop Passport Descriptors.**

It is recommended to request donors that samples be accompanied by the associated data detailed in the FAO/Bioversity Multi-Crop Passport Descriptors (Alercia, Diulgheroff and Mackay, 2015; see Box 1).

✓ **The genebank abides by national, regional and international phytosanitary and any other import regulations and requirements from the relevant authorities.[15]**

When germplasm is moved there is a risk of accidentally introducing plant pests and diseases along with the host plant material. The following steps may help mitigate such risks while ensuring compliance with regulations and requirements:

- for materials from another country, obtaining a phytosanitary certificate from the provider country and an import permit from the relevant authorities in the genebank's country (see IPPC, 2021);
- passing samples through the relevant quarantine/cleaning process before they are incorporated into the genebank collection, if appropriate; and
- regenerating donated accessions with insufficient quantity in containment or in an isolated area, according to the advice of the national phytosanitary authority.

✓ **Donated material is clean of contaminating material (plant debris, soil, non-target seeds) and is as fresh as possible to ensure the material remains viable for as long as possible.**

✓ **Donated material sent to the genebank is prepared, packaged and sent in a way that ensures material arrives intact and viable.[16]**

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.

- In the case of intact fruits and seeds, precautions should be taken to avoid risks of fungal or insect attacks during shipment. Inflated, loosely tied polythene bags can be used to maintain a humid, aerated environment around the fruits/seeds. Bags may then be placed in a rigid container, surrounded by wood shavings or similar compressible material.
- 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.

2.3 Processing of germplasm upon arrival at genebank

✓ **Material arriving at the genebank 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 in a designated reception area. 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.

[15] Standard 6.1.1.
[16] Standard 6.1.4.

- 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.

✓ **Remaining healthy fruits/seeds are disinfected.**[17]

Disinfection is important as fungi can rapidly proliferate under the high humidity conditions required for hydrated storage of recalcitrant seeds. Options for disinfecting seeds include:

- Using hypochlorite solution (NaOCl)
  - soak seeds in 1% NaOCl containing a few drops of wetting agent (e.g. Tween 20/80) for 20-30 minutes;
  - rinse three times with sterile distilled water; and
  - surface-dry with clean paper towels or on filter paper.
- Soaking seeds in warm water ('thermotherapy'; not suitable for all species)
  - immerse seeds in distilled water at 41°C for 2.5 hours;
  - remove floating seeds which could be malformed or insect-damaged; and
  - surface-dry with clean paper towels or on filter paper.

✓ **Immediately after disinfection, decisions are made about what to do with the fruits/seeds received.**

- For recalcitrant species, intact fruits and seeds should be aerated and perhaps transferred to new, clean loosely tied polythene 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).
- For intermediate species, seeds should be immediately prepared for short- to medium-term storage.[18]

✓ **The genebank prepares samples for long-term conservation in the field genebank, via *in vitro* culture and/or through cryopreservation as soon as possible after arrival.**

Due to the short longevity of non-orthodox seeds, hydrated storage of recalcitrant seeds or partially dry, cool storage of intermediate seeds is not effective for the long-term conservation of genetic diversity. Hence, it is important that samples of the original material arriving at the genebank is prepared for long-term maintenance in the field genebank, via *in vitro* culture and/or through cryopreservation.[19] This should be done as soon as possible after the germplasm arrives at the genebank.

[17] Standard 6.1.5.
[18] See section 4.
[19] See section 6.

Figure 3. Summary diagram 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 acquired through collecting missions	- Develop a clear strategy for germplasm collecting missions according to institute's mandate
Germplasm is collected in own or other country	- Develop collecting proposal - Obtain collecting permits - Collect germplasm based on breeding system - Collect from visibly healthy plants - Avoid depleting natural population - Assign collection number for each sample - Use FAO/Bioversity Multi-Crop Passport Descriptors - Obtain any additional information available (farmers; community) - Collect herbarium vouchers/images - Carefully label and avoiding mixing samples - Ensure short interval between collecting and transfer to genebank
Germplasm is packaged and transported to genebank	- Use rigid, insulated packing material - Ensure timely document processing - Check import permit requirements - Use airfreight or courier shipment - Track package if sent by courier
Germplasm is received through donation	- Verify minimum passport data - Ensure identification number for each sample - Abide by national, regional and international phytosanitary and any other import regulations and requirements from the relevant authorities
Samples are received at genebank and added to the collection	- Consult institute's acquisition policy to guide decision to accept material into collection - Check samples and send for processing, including phytosanitary - Conduct viability testing of new material - Multiply seeds if necessary - Assign a unique accession number to sample
Record, validate and upload all acquisition data, including associated metadata	

3. Short-term storage under humid conditions for seeds known to be recalcitrant

The genebank is recommended to have documented policies and/or procedures, as applicable, to ensure incoming fruits/seeds with recalcitrant seed storage behaviour are handled promptly and carefully, such that viability is not compromised before the seeds reach short-term storage[20] or are prepared for longer-term conservation: in field genebanks, *in vitro* culture and/or through cryopreservation.

Recalcitrant seeds cannot be stored for more than a few months to a year in the case of tropical species or perhaps 2-3 years in the case of temperate species. During short-term storage, recalcitrant seeds may be used for distribution, but priority should be given to using the material to prepare for longer-term conservation.

✓ **Seed moisture content is determined, perhaps at an individual seed level.[21]**

The amount of water in seeds plays a fundamental role in determining their physiological response (Hay *et al*., 2023). It is therefore important to determine the moisture content of a sample of seeds, perhaps at an individual seed level and/or for different seed tissues, in particular, embryonic axes. Seed moisture content (*MC*) is usually determined by oven-drying the sample and measuring the change in weight.

MC is determined using the constant low temperature oven method of the International Seed Testing Association (ISTA, 2023a). ISTA recommends using two replicate samples of 5 g each for a moisture content determination, but three replicates may be preferred in a genebank and it may not be possible to use 5 g seed material for each sample. For recalcitrant seeds it may be relevant to test the moisture content of single seeds, in which case, it is recommended that a minimum of 10 individual seeds is tested. It may also be relevant to determine the *MC* of different components of large seeds: embryonic axes and cotyledons and/or endosperm (storage tissues); and/or to chop samples into smaller pieces to ensure moisture can be expelled.

- Weigh the required number of labelled, clean, empty crucibles and then the crucibles with the respective seed sample.
- Place the crucibles (without lids) in an oven at 103°C for 17 ± 1 hours. Allow to cool to ambient temperature in a desiccator containing silica gel.
- Reweigh each crucible + dry seed sample.
- Seed *MC* is often expressed as a percentage of the fresh (starting) weight and calculated as:

$$MC = \frac{f.wt. - d.wt.}{f.wt.} \times 100$$

where *f.wt.* is the starting weight of the seed sample (i.e., not including the crucible weight) and *d.wt.* is the weight of the seed sample after oven drying (not including crucible weight).

- Alternatively, the amount of water in the seeds may be expressed as g H_2O g^{-1} dry weight, i.e.,

$$water\ content\ (g\ H_2O\ g^{-1}\ d.wt.) = \frac{f.wt. - d.wt.}{d.wt.}$$

✓ **Viability of whole seeds or of excised embryos/embryonic axes is tested following optimized and documented procedures, where available.[22]**

It is important to use standard protocols, perhaps developed in-house, so that viability tests are comparable.

[20] See Figure 4 at the end of this section for a summary diagram of the workflow and activities for short-term hydrated storage.
[21] Standard 6.2.2.
[22] Standard 6.3.3.

- Consult online resources for optimized seed germination protocols:
    - the International Seed Testing Association (ISTA) and the Association of Official Seed Analysts (AOSA) publish germination testing procedures, including suggested substrate, optimum temperature regime, and special treatments that may be required to overcome dormancy (ISTA, 2023b); and
    - the Seed Information Database includes details of successful germination protocols for thousands of wild species, including crop wild relatives (SER, INSR and RBGK, 2023).
- If the species is not covered by any of the above sources, search the scientific literature for information on the germination of seeds from species in the same family or genus.
- If predetermined protocols are not available, germination conditions may need to be optimised, but as a starting point, the following may be considered:
    - sow seeds on 1% agar-water, moist filter paper or moist sterilized sand/soil in Petri dishes or clear plastic boxes (depending on seed size, bearing in mind that shoots will grow upwards);
    - use germination temperatures of 25 and 15°C for tropical and temperate species, respectively, or adopt a regime based on climate where the material originated; and
    - use constant dark or a photoperiod of 12 or 8 hours (i.e., 12 hours light/12 hours dark or 8 hours light/16 hours dark), for tropical and temperate species, respectively.
- If testing the viability of embryos/embryonic axes, they should be excised under sterile conditions and sown on appropriate tissue culture media.
- Seeds or excised embryos/embryonic axes should be scored regularly for germination or growth, respectively. This is particularly important for recalcitrant seeds which are often non-dormant. A seed is considered to have germinated when it has produced a morphologically normal seedling capable of developing into a healthy plant.
- A ‘cut’ or ‘squish’ test at the end of the germination test can be used to decide whether seeds are dead (seeds are soft) or possibly still alive and dormant (seeds are firm and tissues have a normal colour).
- Although dormancy is less common in recalcitrant species, if suspected, a tetrazolium test of viability could be carried out on a separate sample of seeds, bearing in mind that a tetrazolium test may give a false-positive result due to the presence of fungi (ISTA, 2023c).

✓ **Clean, disinfected seeds are transferred to clean and labelled airtight storage containers designed to maintain seed moisture content[23].**

The type of container/storage method to use will depend on the size of the seeds being handled and on local facilities. Options include:

- In a large sterile, plastic bucket / box
    - line bucket / box with paper towel wetted with 1% NaOCl;
    - arrange seeds, ideally in a monolayer, on mesh, non-corrodible rack or perforated shelf 100 mm above the base; and
    - attach a layer of dry paper towel to the inside of the lid to prevent build-up of condensation and seal the bucket / box.
- In labelled, inflated, loosely tied polyethylene bags
    - a paper towel or bag can be placed inside the bag to absorb excess moisture.

[23] Standard 6.3.1.

- ✓ **Seeds are stored in temperature-controlled room or incubator at the lowest temperature the seeds will tolerate without cold damage.[24]**
    - Store recalcitrant seeds at 6±2°C, except for species known to show chilling injury such as cacao.
- ✓ **Storage containers are opened regularly to aerate**

  Every 1-2 weeks, open the container, to replenish the oxygen in the container. This should be done in a clean environment and avoiding contamination. In addition,
    - gently mix/turn the seeds by hand (wearing sterile gloves) to improve aeration;
    - visually inspect the seeds for signs of fungal or bacterial contamination[25];
    - remove excess moisture / condensation inside the container with new paper towels; and
    - if using a bucket / box, replace the paper in lid when moist.
- ✓ **Seeds are sampled periodically for moisture content and viability testing[26]**

  Every 4-8 weeks, depending on the number of seeds in storage, take samples for moisture content and viability testing. The following scenarios may be observed:
    - significant decline in both moisture content and viability, probably because high RH was not maintained and, consequently, the seeds have lost moisture and died;
    - significant decline in viability, indicating that the end of the useful storage period has been reached.

  In both situations, the remaining material should be discarded according to normal biological waste-management procedures.
    - If only moisture content has declined, adding wet material (e.g., wet paper towel) to the storage container may prevent further drying of the seeds.
- ✓ **All cleaning/disinfecting, storage and monitoring data, including associated metadata, are recorded, validated and uploaded to the genebank information management system.**

  Data to consider include accession location (room/incubator and position), number of seeds per location, moisture content, storage pretreatment, date of inclusion in the collection and, when sampling, state of the seeds, how many are sampled, etc.

  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 and minimizes human error.

[24] Standard 6.3.1.
[25] Standard 6.3.3.
[26] Standard 6.3.3.

Figure 4. Summary diagram of the workflow and activities for short-term storage under humid conditions for seeds known to be recalcitrant

Seed moisture content is determined, perhaps at an individual seed level	- Determine *MC* by oven-drying the sample and measuring the change in weight following established protocols
Viability of whole seeds or of excised embryos/embryonic axes is tested following optimized and documented procedures	- Consult online resources for optimized seed germination protocols - If not available, search scientific literature for information on species in the same genus or family and optimize as necessary
Clean, disinfected seeds are transferred to clean and labelled airtight storage containers designed to maintain seed moisture content and stored	- Select the type of container/storage method depending on the size of the seeds being handled and on local facilities - Store seed in temperature-controlled room or incubator at the lowest temperature the seeds will tolerate without cold damage
Storage containers are opened regularly to aerate	- Open the container every 1-2 weeks to replenish the oxygen in the container.
Seeds are sampled periodically for moisture content and viability testing	- Take samples for moisture content and viability testing every 4-8 weeks, depending on the number of seeds in storage - Add wet material (e.g., wet paper towel) to the storage container if moisture content has declined - Discard seed that have significant decline in viability or have died, using normal biological waste-management procedures
Record, validate and upload all short-term storage data, including associated metadata	

4. Short- to medium-term storage of seeds known to be intermediate

The genebank is recommended to have documented policies and/or procedures, as applicable, specific for the storage of intermediate seeds.[27]

So-called intermediate seeds fall somewhere in the seed storage behaviour continuum between orthodox and recalcitrant seeds. They usually tolerate some drying and some tolerate drying as much as orthodox seeds but their response to low temperature is anomalous in that they lose viability faster at -18°C than expected by extrapolating longevity at higher temperatures. This has led to two sub-categories: 'desiccation-intermediate' seeds which tolerate partial drying, and 'low-temperature intermediate' seeds which may tolerate drying to lower moisture content but show poor germination after a relatively short period (compared with orthodox seeds) of storage at -18°C.

The longevity of intermediate seeds varies, but in general, under optimized conditions, high levels of viability can be maintained for longer than recalcitrant seeds, but not as long as most orthodox seeds.

If seeds of a particular species are known to be intermediate, the optimum storage conditions (lowest safe moisture content and lowest safe temperature) may have already been determined and these conditions should be adopted. Otherwise, a procedure is presented below to determine the optimum storage conditions (Table 2).

✓ **Seeds are cleaned and sorted.**

Seeds are extracted from dry fruits, pods and spikes and cleaned to remove debris and broken, damaged or diseased seeds.

✓ **Seed moisture content (MC) and/or water activity is determined.**

It is important to understand the water status of the seeds, either by measuring seed moisture content[28] and/or seed water activity if a water activity meter is available.

- Measure water activity according to the manufacturer's instructions for using the water activity meter.
  - It is important to fill the measuring chamber as much as possible.
  - Measurement should be at a constant temperature and the measurement temperature recorded.

✓ **Initial viability (germination) is tested following optimized and documented procedures, where available.**

It is important to use standard protocols, perhaps developed in-house, so that viability tests are comparable.

- Consult online resources for optimized germination protocols:
  - the International Seed Testing Association (ISTA) and the Association of Official Seed Analysts (AOSA) publish germination testing procedures, including suggested substrate, optimum temperature regime, and special treatments that may be required to overcome dormancy (ISTA, 2023b); and
  - the Seed Information Database includes details of successful germination protocols for thousands of wild species, including crop wild relatives (SER, INSR and RBGK, 2023).
- If the species is not covered by any of the above sources, search the scientific literature for information on the germination for species in the same family or genus.
- If predetermined protocols are not available, germination conditions may need to be optimised, but as a starting point, the following may be considered:

[27] See Figure 5 at the end of this section for a summary diagram of the workflow and activities for storage of intermediate seeds.
[28] See section 3.

- sow seeds on 1% agar-water, moist filter paper or moist sterilized sand/ soil in Petri dishes or clear plastic boxes (depending on seed size, bearing in mind that shoots will grow upwards);
- use germination temperatures of 25 and 15°C for tropical and temperate species, respectively, or adopt a regime based on climate where the material originated;
- use constant dark or a photoperiod of 12 or 8 hours (i.e., 12 hours light/12 hours dark or 8 hours light/16 hours dark), for tropical and temperate species, respectively.

- Seeds should be scored regularly for germination. This is particularly important for recalcitrant seeds which are often non-dormant. A seed is considered to have germinated when it has produced a morphologically normal seedling capable of developing into a healthy plant.
  - Construction of a germination progress curve (cumulative percentage germination plotted against time from sowing) enables an evaluation of the speed of germination, which can be used as an indicator of seed lot vigour. When seed physiological quality is compromised, germination is often slower.
- A 'cut' or 'squish' test can be used to decide whether seeds are dead (seeds are soft) or possibly still alive and dormant (seeds are firm and tissues have a normal colour) (RBGK, 2022b).
- If dormancy is suspected, a tetrazolium test of viability could be carried out on a separate sample of seeds (ISTA, 2023c).

✓ **Seeds are dried to the lowest safe moisture content or RH.**

Seeds can be dried in different ways, for example, by placing seeds over conditioned silica gel or equilibrating seeds over saturated or non-saturated salt solutions or in a drying chamber or room (Hay *et al*., 2023). Seeds should be dried at 25°C for seeds from tropical species and 15°C for seeds from temperate species.

- In the case of desiccation-intermediate seeds where an optimum storage moisture content is known and to avoid drying below this safe moisture content, the following formula can be used, measuring the total sample mass at regular intervals:

$$Target\ sample\ mass\ (g)\ = initial\ sample\ mass\ (g) \times \frac{100 - initial\ MC}{100 - target\ MC}$$

- For intermediate seeds for which the optimum storage moisture content is not known, in the first instance, it is recommended to dry seeds at 50% RH.
- Seeds should not be left in the drying/equilibration environment longer than necessary as ageing may be accelerated leading to decline in quality.

✓ **After drying, samples are packaged, minimizing the risk that seed moisture content changes further, in clearly labelled airtight containers.**

Sealing samples in airtight containers ensures that the moisture content of the seeds does not change during storage. It is also important that the moisture content of the seeds does not change during packing by working efficiently and not exposing seeds to ambient conditions for more than a few minutes. Additional best practices include:

- filling the container to minimize the air gap above the seeds helps to prevent changes in seed moisture content (ideally keep a range of container sizes to suit the volume of seeds for different accessions);
- using both an outer and an inner label (preferably barcoded) for each sample to ensure that the material is properly identified.

✓ **Samples are stored at the lowest safe temperature.**

Accessions of intermediate seeds may be stored in refrigerators or incubators. If the lowest safe temperature is not known, then seeds should be stored at 6±2°C.Best practices include:

- avoiding opening refrigerators/incubators during any periods of power loss; and
- minimizing the time spent at higher temperature (but allow containers to equilibrate to the external temperature before opening to avoid condensation forming on the cold seeds).

✓ **Viability monitoring is carried out at regular intervals.**

- Seed viability should be monitored on a sample of seeds every 6-24 months.
    - Some intermediate seeds may be susceptible to imbibition damage and hence it may be helpful to allow seeds to take up moisture in a humid environment (over water in a sealed container) before sowing.

If viability declines below 50-70% and the accession has not already been conserved using an alternative method of *ex situ* conservation (in a field genebank, *in vitro* and/or cryopreservation), consider whether the accession should be regenerated (FAO, 2022a, section 5) or replaced with a new collection from the same location, if possible. If the latter action is taken, the new sample should be considered and handled as a new accession. The original accession should be discarded and labeled as historical in the genebank information management system.

✓ **Optimum storage conditions for those species where the information is unknown is determined using established procedures.**

If seeds are thought to be intermediate but optimal storage conditions (moisture content × temperature) have not been determined, a designed experiment may be carried out whereby seeds are dried to different levels (i.e. at different relative humidities, RH) and stored at different temperatures:

- dry seeds to different moisture levels (Table 2)
    - dry seeds of tropical origin at 25°C and seeds of temperate origin at 15°C;
- seal inside airtight containers, for example, aluminium foil laminate pouches;
- store seeds at each moisture level at each of a range of storage temperatures (Table 2)
    - due to the specific composition of seeds of particular species, longevity may be compromised at -20°C, hence it is a good idea to try other temperatures below 0°C if feasible;
- remove a sample of seeds from each storage environment after 3 and 6 months, and 1, 2, 3 and 5 years to test seed moisture content and viability;
- comparison of the viability data should indicate the optimum storage conditions.

Table 2. Combination of storage regimes to use to determine the optimum storage environment for orthodox seeds (adapted from Hong and Ellis, 1996; Walters *pers. comm.*).

		Store seeds hermetically at the indicated temperature (°C)					
		35 (optional)	20	6*	-20*	-80 (optional)	LN2
Dry seeds at RH (%)	50						
	30		Test seed moisture content and viability after 3 and 6 months, and 1, 2, 3 and 5 years				
	15						

*These test storage temperatures should be prioritized if the number of seeds available is limited.

- ✓ **All cleaning/disinfecting, storage and monitoring data, including associated metadata, are recorded, validated and uploaded to the genebank information management system.**

  Data to consider include accession location (room/incubator and position), number of seeds per location, moisture content, storage pretreatment, date of inclusion in the collection and, when sampling, state of the seeds, how many are sampled, etc.

  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 and minimizes human error.

Figure 5. Summary diagram of the workflow and activities for short- to medium-term storage of seeds known to be intermediate

Seeds are cleaned and sorted	- Extracted seeds from dry fruits, pods and spikes and cleaned to remove debris and broken, damaged or diseased seeds
Seed moisture content is determined, perhaps at an individual seed level	- Determine *MC* by oven-drying the sample and measuring the change in weight following established protocols
Initial viability (germination) is tested following optimized and documented procedures, where available	- Use standard protocols, perhaps developed in-house, so that viability tests are comparable - If unavailable search the scientific literature and for species in the same genus or family and optimize as necessary
Seeds are dried to the lowest safe moisture content or RH	- Place seeds over conditioned silica gel or equilibrate over saturated or non-saturated salt solutions or in a drying chamber or room - Determine appropriate storage moisture through testing or literature - Dry seeds at 25°C for seeds from tropical species and 15°C for seeds from temperate species - Avoid leaving seeds in drying environment too long
After drying, samples are packaged, minimizing the risk that seed moisture content changes further, in clearly labelled airtight containers	- Seal samples in airtight containers to ensure that the moisture content of the seeds does not change during storage - Fill the container to minimize the air gap above the seeds helps to prevent changes in seed moisture content - use both an outer and an inner label for each sample to ensure that the material is properly identified.
Samples are stored at the lowest safe temperature	- Store accessions of intermediate seeds in refrigerators or incubators. - If the lowest safe temperature is not known, store seeds at 6±2°C.
Viability monitoring is carried out at regular intervals	- Monitor seed viability of a sample of seeds every 6-24 months - Set viability threshold at 50-70% - Use alternative conservation method or regenerate if viability falls below threshold
Record, validate and upload all short-to medium-term storage data, including associated metadata	

5. Testing of seeds with unknown but suspected non-orthodox seed storage behaviour

The genebank is recommended to have documented policies and/or procedures, as applicable, specific for the testing of seeds with unknown but suspected non-orthodox seed storage behaviour.[29]

The genebank is encouraged to do research to characterize seed storage behaviour when handling a species suspected to produce non-orthodox seeds.

Testing of storage behaviour should be carried out on intact fruits/seeds, depending on the unit of dispersal, as soon as possible after harvest, by assessing the response to drying[30] and storage at low temperatures. The material should not be allowed to dry before testing is conducted. Initial cleaning and sorting may be necessary. During experimentation, cleaned seed samples [not used for testing] should be stored under conditions that do not allow any dehydration or hydration[31] until a decision can be made about how to proceed: treat as orthodox; treat as intermediate; or treat as recalcitrant.

✓ **Intact fruits and seeds should be maintained in temporary humid storage.[32]**

- Intact fruits and seeds should be aerated and perhaps transferred to new, clean loosely-tied polythene bags, or sterile plastic buckets/boxes, while still maintaining the moisture content as far as possible. may be at the same temperature as final storage:
  - Temporary storage should be at 6±2°C.

✓ **Predict seed storage behaviour based on seed/fruit structure.**

Before proceeding with the desiccation tolerance test, an initial prediction of non-orthodox behaviour (desiccation-sensitivity) can be made based on seed/fruit structure (Daws et al., 2006):

- measure the individual seed mass (g) for a sample of seeds (10 or more individual seeds) as soon as possible after harvest and calculate the mean seed mass (*SM*);
- dissect each seed, to remove the outer layer (seed coat ± fruit endocarp, in some cases), measure the mass of the outer layer material for each seed and calculate the individual seed coat ratio, *SCR*, as $mass\ (g) of\ covering\ structures/total\ seed\ mass\ (g)$.
- The probability of recalcitrant storage behaviour (desiccation sensitivity) is calculated as:

$$P(\text{desiccation-sensitivity}) = \frac{e^{3.269-9.974SCR+2.156\log_{10} SM}}{1+e^{3.269-9.974SCR+2.156\log_{10} SM}}$$

✓ **Seed moisture content and/or water activity is determined, perhaps at an individual seed level.[33]**

It is important to understand the water status of the seeds, either by measuring seed moisture content[34] and/or seed water activity if a water activity meter is available.

- Measure water activity according to the manufacturer's instructions for using the water activity meter.
  - It is important to fill the measuring chamber as much as possible.
  - It may be necessary to cut large seeds into small pieces so that the measuring chamber can be filled.

[29] See Figure 6 at the end of this section for a summary diagram of the workflow and activities for determining seed storage behaviour.
[30] Standard 6.2.1.
[31] Standard 6.2.4.
[32] Standard 6.2.4.
[33] Standard 6.2.2.
[34] See section 3.

- Measurement should be at a constant temperature and the measurement temperature recorded.

✓ **Viability of whole seeds or of excised embryos/embryonic axes is tested following optimized and documented procedures, where available.[35]**

It is important to use standard protocols, perhaps developed in-house, so that viability tests are comparable.

- Consult online resources for optimized germination protocols:
  - the International Seed Testing Association (ISTA) and the Association of Official Seed Analysts (AOSA) publish germination testing procedures, including suggested substrate, optimum temperature regime, and special treatments that may be required to overcome dormancy (ISTA, 2023b); and
  - the Seed Information Database includes details of successful germination protocols for thousands of wild species, including crop wild relatives (SER, INSR and RBGK, 2023).
- If the species is not covered by any of the above sources, search the scientific literature for information on the germination of seeds from species in the same family or genus.
- If predetermined protocols are not available, germination conditions may need to be optimised, but as a starting point, the following may be considered:
  - sow seeds on 1% agar-water, moist filter paper or moist sterilized sand/soil in Petri dishes or clear plastic boxes (depending on seed size, bearing in mind that shoots will grow upwards);
  - use germination temperatures of 25 and 15°C for tropical and temperate species, respectively, or adopt a regime based on climate where the material originated;
  - use constant dark or a photoperiod of 12 or 8 hours (i.e., 12 hours light/12 hours dark or 8 hours light/16 hours dark), for tropical and temperate species, respectively.
- If testing the viability of embryos/embryonic axes, they should be excised under sterile conditions and sown on appropriate tissue culture media.
- Seeds or excised embryos/embryonic asex should be scored regularly for germination or growth. Different valuation criteria are used for germination: production of a morphologically normal seedling capable of developing into a healthy plant is probably the most useful criterion.
  - Construction of a germination progress curve (cumulative percentage germination plotted against time from sowing) enables an evaluation of the speed of germination, which can be used as an indicator of seed lot vigour. When seed physiological quality is compromised, germination is often slower.
- A ‘cut’ or ‘squish’ test can be used to decide whether seeds are dead (seeds are soft) or possibly still alive and dormant (seeds are firm and tissues have a normal colour).
- If dormancy is suspected, a tetrazolium test of viability could be carried out on a separate sample of seeds (ISTA, 2023c).

✓ **Test for seed storage behaviour based on response to drying and storage.[36]**

If plenty of seeds are available, seed storage behaviour can be determined based on a modified version of the protocol of Hong and Ellis (1996). Briefly, this is as follows (Figure 6):

- Determine initial seed viability and moisture content and/or water activity.

[35] Standard 6.2.3.
[36] Standard 6.2.1.

- Dry seeds at 50% RH; re-test viability (and optionally, moisture content and/or water activity). If seeds are not viable, they are probably recalcitrant.
- Dry seeds at 15% RH; re-test viability (and optionally, moisture content and/or water activity). If seeds are not viable, they are probably desiccation-intermediate.
- Place seeds dried at 15% RH in airtight containers (e.g. aluminium foil laminate packets) and place at -20°C for up to five years; re-test viability (and optionally, moisture content and/or water activity) after 3 and 6 months, and 1, 2, 3 and 5 years. If seeds are not viable, they are probably low temperature-intermediate; if high viability has been maintained, orthodox seed storage behaviour can be concluded.

For this test, seeds can be dried in different ways, for example, using silica gel, saturated or non-saturated salt solutions, or in a dedicated drying chamber or room, if available (Hay *et al*., 2023). Seeds should be dried at 25°C for seeds from tropical species and 15°C for seeds from temperate species.

✓ **Test for non-orthodox seed storage behaviour based on response to drying when seeds numbers are limited.**

When seed numbers are limited, a modified version of the above protocol can be conducted in which seeds are only dried at the lower RH (15%) and low numbers are used for moisture content determination (5-10, as single seeds) and viability testing (30-40, sown across two or more plates / germination boxes) (Pritchard *et al*., 2004):

Pritchard *et al*. (2004) also included a control stored at high humidity and tested for viability at the same time as the dried seeds. If germination of these seeds is higher than the germination recorded in the initial viability test, the seeds may have matured during storage.

✓ **Confirm non-orthodox seed storage behaviour.**

Since the conservation of species with recalcitrant seed storage behaviour is more complicated and costly, it is important to avoid erroneously concluding non-orthodox seed storage behaviour (Hong and Ellis, 1996). Mistakenly categorizing seeds as recalcitrant or intermediate may be because seeds have been stored under suboptimal conditions; this will compromise their longevity. A number of considerations should be taken into account:

- ensure seeds are mature at harvest
    - orthodox seeds acquire desiccation tolerance as they develop and hence, if harvested too early, lose viability upon drying;
    - conversely, orthodox seeds that are not dispersed from the plant and harvested late or which are collected from the ground may have aged and lost viability or desiccation tolerance if germination has commenced.
- make sure the drying process is efficient, by arranging in a monolayer if possible, and turning seeds regularly as seeds may lose viability during drying, especially when their initial MC is high.
- it is important that viability tests are not stopped too early
    - a cut test at the end of a germination test can be used to evaluate whether seeds are likely to be dead or dormant.
- dry seeds of some species are prone to imbibition damage, whereby a rapid influx of water damages cell membranes and reduces viability
    - an additional sample of dry seeds should be imbibed slowly in a humid environment (e.g. over water) before sowing for germination.
- if possible, test more than one seed lot, perhaps from different locations since desiccation tolerance can vary for seed lots harvested in different locations (Daws *et al*., 2006).

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

Data to consider include accession location (room/incubator and position), number of seeds per location, moisture content, storage pretreatment, date of inclusion in the collection and, when sampling, state of the seeds, how many are sampled, etc.

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 and minimizes human error.

Figure 6. Protocol to determine seed storage behaviour.

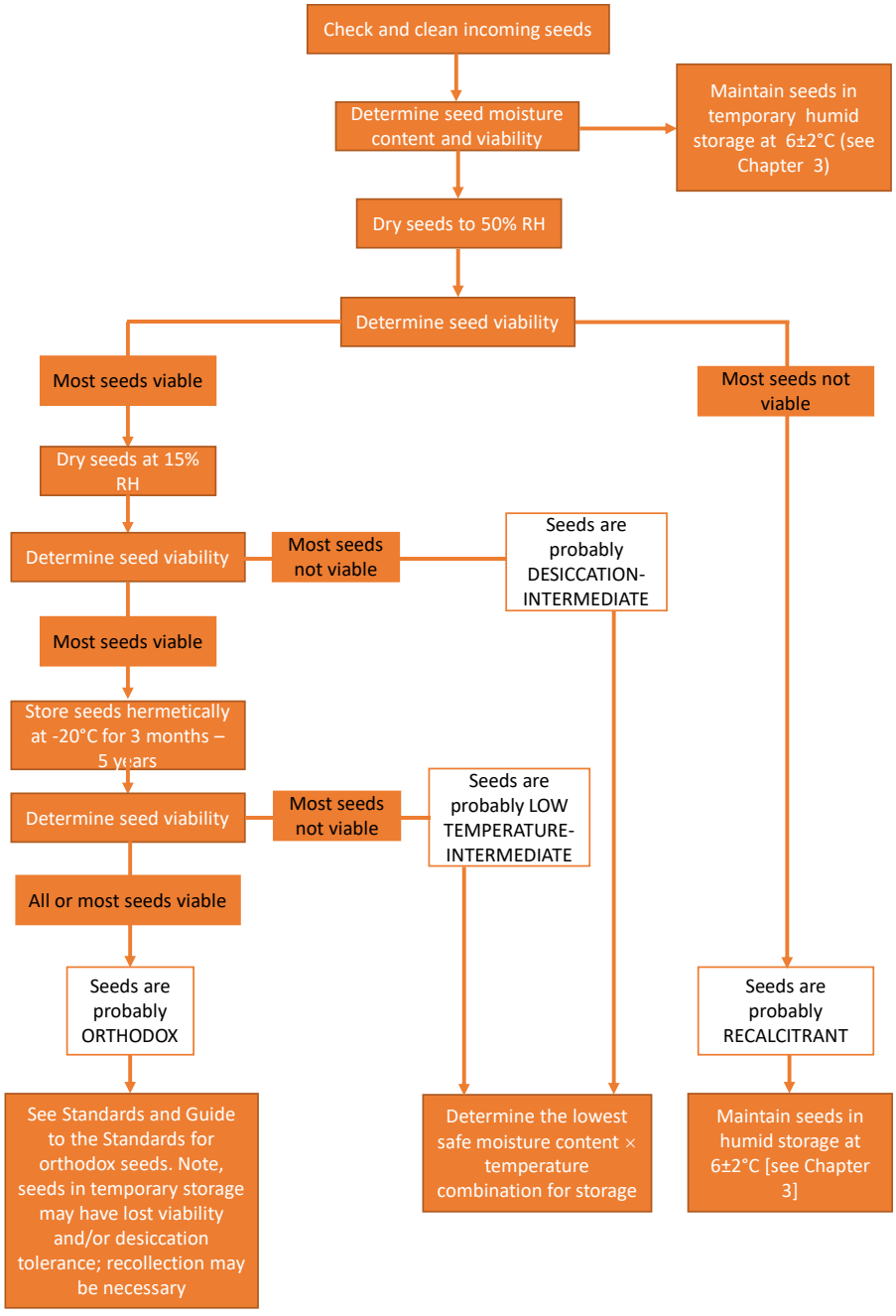


Figure adapted from Hong and Ellis (1996).

6. Preparing material for long-term conservation.

The genebank is encouraged to have documented policies and/or procedures, as applicable, and facilities in place, for the longer-term *ex situ* conservation of species with recalcitrant or intermediate seed storage behaviour.[37] Fruit/seed lots arriving at the genebank and known or found (see section 5) to have recalcitrant or intermediate storage behaviour should be sampled as soon as possible after arrival. These samples are used to prepare material for long-term conservation in the field genebank, *in vitro* and/or in cryopreservation. Cryopreservation of whole small-sized intermediate seeds and excised embryos or embryonic axes of non-orthodox seeds is also considered as a backup method for the genebank. Safety duplication of accessions maintained in field, *in vitro* and/or cryopreservation collections are described in detail in FAO 2022 a, b and c.

6.1 Preparation of non-orthodox seeds for a field genebank.

A common method to conserve germplasm of species producing recalcitrant or intermediate seeds is to maintain them as live plants in field genebanks. Detailed guidance on the workflow of a field genebank is available (FAO, 2022b).

✓ **Seeds are used as soon as possible after arrival at the genebank.**

Depending on the species, it may be possible to use seedlings from the laboratory viability testing (see sections 3-5); these should be carefully transferred to soil in clearly labelled seedling trays/pots. Otherwise, a fresh sample should be taken from the seeds in temporary storage and sown directly in soil in clearly labelled seedling trays/pots.

- It may be beneficial to surface-disinfect seeds before sowing.[38]

✓ **A sufficient number of individuals are planted to capture genetic diversity and ensure the safety of each accession.**

To determine the number of individuals to be planted per accession it will be necessary to differentiate between annual, biennial and perennial species. [39]

- The number of plants needs to be sufficiently large to represent the within accession diversity.[40]
- For dioecious species, it is important to plant a suitable number of male/female parents.

✓ **Appropriate planting and cultural practices are used to provide optimum conditions for plant establishment.**

Field establishment requires use of appropriate spacing of plants, planting time, shading, irrigation, weed control, etc. based on the species and local conditions.[41]

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

6.2 Preparation of non-orthodox seeds for *in vitro* culture.

An alternative approach for conserving germplasm of species producing recalcitrant and/or intermediate seeds is to maintain them as live plantlets via in vitro cultivation Note, the action steps described here only apply to the excision of embryos / embryonic axes from non-orthodox seeds already at the genebank. Detailed guidance on the workflow of an *in vitro* genebank is available (see FAO, 2022c).

[37] See Figure 7 at the end of this section for a summary diagram of the workflow and activities for preparation of non-orthodox material for long term conservation.
[38] See section 2.3.
[39] See FAO, 2022b, section 4.
[40] Guidelines can be extrapolated from germplasm collection practices.
[41] See FAO, 2022b, section 4.

✓ **Seeds are used as soon as possible after arrival at the genebank.**

Immature axes are a good starting material for the establishment of embryogenic cultures.

✓ **The embryos / embryonic axes are excised from a sufficient number of seeds to capture genetic diversity and ensure the safety of each accession.**

✓ **Embryos/embryonic axes are excised under sterile conditions.**

Only healthy-looking fruits/seeds with no signs of disease or damage should be used. The fruits/seeds should be surface sterilized[42] and the entire operation should be carried out under sterile conditions.

- 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.
- Excised embryos / embryonic axes should be immediately placed in antioxidant solution and then in sterile medium in *in vitro* tubes (please see FAO, 2022c for guidance on *in vitro* culture).
    - Examples of antioxidant solution include 50 mg / L ascorbic acid in water or liquid culture medium; 50 mg / L polyvinylpolypyrrolidone (PVP); or 10 mg / L citric acid.

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

6.3 Preparation of excised embryos or embryonic axes of non-orthodox seeds for cryopreservation.

Cryopreservation of embryos or embryonic axes from large recalcitrant or intermediate seeds is often considered a method for backing up germplasm, especially for materials that would be expensive and labour intensive using other means (field/*in vitro* culture). However, it is still largely experimental, with the focus on method development to determine e.g., optimum water content and freezing and warming rates, rather than routine conservation.

The basic steps required for the preparation of embryonic axes from large non-orthodox seeds for cryopreservation are given below; detailed guidance on the genebank workflow for cryopreservation of germplasm is available (see FAO, 2025).

✓ **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).

✓ **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. The fruits/seeds should be surface sterilized[43] and the entire operation should be carried out under sterile conditions.

- 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.

[42] See section 2.3.
[43] See section 2.3.

- An example of antioxidant solution is 50 mg / L ascorbic acid in water or liquid culture medium.

✓ **The number of embryos/embryonic axes to excised is determined**

Information to consider includes 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 embryos/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 (Ballesteros *et al.*, 2021).
- Encapsulation-dehydration, vitrification, or encapsulation-vitrification are species-specific procedures developed for cryopreserving non-orthodox seed embryos or embryonic axes (Ballesteros *et al.*, 2021).

✓ **Embryonic axes are stored in cryovials at LN temperatures**

- Transfer embryos/embryonic axes to cryovials and immerse in liquid nitrogen.
- Store cryovials in racks in liquid nitrogen vapour phase.

6.4 Preparation of whole intermediate seeds for cryopreservation

Cyopreservation may be preferable for intermediate seeds due to their short longevity at relatively high storage temperatures. Intermediate seeds may be cryopreserved either as whole seeds or as excised embryos (Section 6.3), depending on the seed size. Further information on the cryopreservation of intermediate seeds is available (FAO, 2025).

✓ **Small intermediate seeds are stored at optimum moisture for cryopreservation.**

- Equilibrate seeds at 50% RH at 15-20°C. This can be in a room with controlled relative humidity and temperature or by placing seeds in chambers with saturated salt solutions.
- If sufficient seeds are available, seed moisture content may be determined.[44]
- Pack samples in cryovials.
  - Seed size may be a limiting factor to the number of seeds that can be conserved in vials.
  - Samples may be stored in multiple cryovials, also to allow removal of individual vials for monitoring and distribution.
- Store cryovials in racks in liquid nitrogen vapour phase.

[44] See section 3.

Figure 7. Summary diagram of the workflow and activities for preparing material for long-term conservation

Preparation of non-orthodox seeds for a field genebank	
Seeds are used as soon as possible after arrival at the genebank	- Transferred seedlings from viability testing to soil in clearly labelled seedling trays/pots, depending on the species - Alternatively, plant seed directly in soil in clearly labelled seedling trays/pots
A sufficient number of individuals are planted to capture genetic diversity and ensure the safety of each accession	- Plant enough seeds to represent the within accession diversity, differentiating annual, biennial and perennial species
Appropriate planting and cultural practices are used to provide optimum conditions for plant establishment	- Use spacing of plants, planting time, shading, irrigation, weed control, etc. appropriate for the species and local conditions.
Preparation of non-orthodox seeds for *in vitro* culture	
Seeds are used as soon as possible after arrival at the genebank	- Use immature axes for the establishment of embryogenic cultures
The embryos / embryonic axes are excised from a sufficient number of seeds to capture genetic diversity and ensure the safety of each accession	- Use enough seeds to represent the within accession diversity, differentiating annual, biennial and perennial species
Embryos/embryonic axes are excised under sterile conditions	- Use healthy-looking fruits/seeds with no signs of disease or damage - Surface sterilize fruits/seeds and carry out all procedures under sterilized conditions - Use optimized procedures for excision of the explant - Immediately place excised embryos / embryonic axes in antioxidant solution and then in sterile medium in *in vitro* tubes
Preparation of excised embryos or embryonic axes of non-orthodox seeds for cryopreservation	
Seeds are subjected to initial viability testing following optimized and documented procedures	- Use standard protocols so that viability monitoring tests are comparable
Embryos/embryonic axes are excised from seeds as soon as possible after the seeds arrive at the genebank	- Use healthy-looking fruits/seeds with no signs of disease or damage - Surface sterilize fruits/seeds and carry out all procedures under sterilized conditions - Use optimized procedures for excision of the explant - Immediately place excised embryos / embryonic axes in antioxidant solution
The number of embryos/embryonic axes excised is determined	- Consider 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-	- Use species specific protocols

dehydration or vitrification or encapsulation-vitrification	
Embryonic axes are stored in cryovials at LN temperatures	- Transfer embryos/embryonic axes to cryovials - Store cryovials in racks in liquid nitrogen vapour phase
Preparation of whole intermediate seeds for cryopreservation	
Small intermediate seeds are stored at optimum moisture for cryopreservation	- Equilibrate seeds at 50% RH at 15-20°C - Determine seed moisture content if sufficient seeds are available - Pack samples in packets cryovials - Store in racks or other containers in liquid nitrogen vapour phase
Record, validate and upload all preparation for long-term conservation data, including associated metadata	

7. Documentation

The genebank is recommended have a documented policy and/or procedure, as applicable, for managing genebank data and information, including data-sharing guidelines.[45]

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

The genebank information system is ideally designed to manage all the data and information generated relating to all aspects of the conservation and use of germplasm, including passport, short-term hydrated storage of recalcitrant seed, short- and medium-term storage of intermediate seed, testing for non-orthodox behavior and assessing moisture content, preparing material for long-term conservation, distribution, and all management data and metadata. Built-in automated tools for checking inventory and propagule/plantlet health, and flagging accessions requiring regeneration, should be available.

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). Other systems include the AVRDC Vegetable Genetic Resources Information System (AVGRIS) (AVRDC, 2021), the German Genebank Information System (GBIS) (GBIS/I, 2021) and Alelo developed by the Brazilian Agricultural Research Corporation (Embrapa) (Embrapa, 2021).

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

Recording the passport data of accessions using the FAO/Bioversity Multi-Crop Passport Descriptors (Alercia, Diulgheroff and Mackay, 2015) facilitate data exchange and comparison of accessions across different countries and institutions. Passport data are ideally available for all accessions in the genebank collection.[46]

A unique and permanent accession number is a key element of proper documentation and identification. The voluntary use of Digital Object Identifiers (DOIs) (Alercia, Diulgheroff and Mackay, 2015; FAO, 2021a) is an additional option for information sharing across different information systems and different communities but cannot replace the assignment of the genebank's unique and permanent accession number.

✓ **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.[47]**

Having trained staff responsible for data recording and data entry in close collaboration with documentation officers and germplasm collection curators supports quality control. It would be useful to have staff members that are assigned specific responsibility for managing the genebank information management system, including keeping data up to date at all times. Validation of data by genebank curators and documentation officers before being uploaded into the genebank information management system is recommended.

[45] See Figure 8 at the end of this section for a summary diagram of the workflow and activities for documentation.
[46] Standard 6.6.1.
[47] Standard 6.6.3.

✓ **Data are publicly available in a search-query database, if possible.**

Publishing data on the genebank holdings increases opportunities for use of germplasm and therefore gives value and prestige to genebanks. It may not be possible for all genebanks to maintain a web portal for external access to collection information. An option is to provide information through Genesys, an international global portal managed by the Global Crop Diversity Trust (Crop Trust, 2021). Genesys allows accession data from genebanks around the world to be shared and facilitates the ordering of germplasm. It includes accession-level passport, characterization and evaluation data as well as environmental information associated with accession collecting sites. Another option for making the passport data of genebank accessions publicly accessible is provided by the FAO World Information and Early Warning System on Plant Genetic Resources for Food and Agriculture (WIEWS) (FAO, 2021d). By serving as the data repository for the plant indicator of Target 2.5 of the Sustainable Development Goals (United Nations, 2021), WIEWS stores and publishes accession-level passport data for the largest global inventory of *ex situ* collections (FAO, 2021e).

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

Figure 8. Summary diagram of the workflow and activities for documentation

Documentation	
A suitably designed genebank information management system is used, for example, GRIN-Global Community Edition	
International data standards are adopted for consistency in data sharing	- 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 update Seed viability and moisture content data are recorded	- Use built-in automated tools to check inventory and viability, and flag accessions requiring regeneration
Germplasm orders, distribution information and user feedback are documented	
Transfer to long-term conservation data are recorded	
Data are publicly available, possibly though a search–query database	- Consider uploading to Genesys
Data are duplicated (backed up) at regular intervals and stored at a remote site for security reasons	

8. Distribution of non-orthodox seeds

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.[48]

✓ **The genebank complies with national, regional and international regulations and agreements.[49]**

The process of germplasm distribution is governed by national and international regulations. The genebank should communicate with the appropriate designated authorities when there are questions regarding germplasm distribution. The following information should assist in ensuring compliance:

- The genebank should communicate with the Secretary of the Treaty or a National Focal Point or other designated authority if other countries are involved in germplasm distribution.
- If the genebank's country is a signatory to the Treaty and germplasm of crops or species listed under Annex 1 of the Treaty (FAO, 1995) are being distributed for the intended uses covered by the Treaty (i.e. research, breeding and training for food and agriculture), it is necessary to use an SMTA (FAO, 2021b; c).
- If the genebank's country is not a Contracting Party to the Treaty or if the germplasm is not covered under Annex 1, it is recommended that an agreement be reached with the recipient on the terms and conditions of germplasm distribution – covering, for example, the use and onward sharing of the material or its derivatives, data reporting, etc. An MTA is usually used (e.g. AVRDC, 2012), though an SMTA could also be used.

✓ **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.

✓ **Arrangements are made with competent authorities or agents (i.e. the country's National Plant Protection Organization) to inspect or test the material in order to ensure compliance with the regulations of the importing country and to issue the relevant phytosanitary certificate.**

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

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

Correctly labelled samples, preferably with computer-produced labels to reduce transcription errors, should be placed both outside and inside each seed packet to ensure that the material is properly identified.

[48] See Figure 9 at the end of this section for a summary diagram of the workflow and activities for distribution of germplasm.
[49] Standard 6.6.1.

✓ **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).**[50]

Consider scanning documents and sending them by e-mail, or sending hard copies by mail, prior to the dispatch of the germplasm. Items of documentation to consider include:

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

✓ **The delivery of the germplasm and its condition on arrival at its destination is checked by following up with the recipient.**

It is recommended to track the shipment and follow up with the recipient on the status and performance 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, number of seeds per sample and/or weight; 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 bar-code labels and barcode readers facilitates accession management and minimizes human error.

8.1 Distribution of recalcitrant seeds in humid storage

✓ **A policy is in place for the number of seeds to distribute for any given species.**

Decisions about how many seeds to supply depends on the total number of seeds in storage. In the case of recalcitrant seeds, for which, in general, relative few seeds are likely to be conserved in hydrated storage, the number of seeds to distribute may by quite low (<30-50). Nonetheless, efforts should be made to ensure the distributed sample captures the genetic diversity of the seeds in storage. The projected shelf-life may also influence the sample size for distribution; it is recommended to distribute material that is already in long-term storage conditions to other institutes before viability declines below 50 percent.

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

Ensure that the material reaches the germplasm requestor in good condition, bearing in mind the time needed for document processing, duration of shipment, transit time and transit conditions (high or low temperatures). The use of packing and shipping guidelines/recommendations similar to those utilized for acquisition is recommended (see acquisition section).

8.2 Distribution of intermediate seeds

✓ **A policy is in place for the number of seeds to distribute for any given species.**

For most species, a sample of 50–200 viable seeds would be supplied for those accessions with sufficient seeds.

- For accessions with too few seeds at the time of the request, and in the absence of a suitable alternative accession, samples are supplied after regeneration, based on a renewed request. For some species and for some uses, a smaller number of seeds may be provided upon agreement with the requestor.

[50] Standard 6.6.2.

- If feasible, consider the distribution of samples with a mutually signed regeneration agreement. In this case, the requesting institute should have the necessary technical capacity and regeneration should be carried out under the supervision of staff from the genebank according to the genebank's protocols.

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

Ensure that the material reaches the requesting institution in good condition, bearing in mind the time needed for document processing, duration of shipment, transit time and transit conditions (high or low temperatures).

- Containers/packets of seeds should be allowed to equilibrate at room temperature before opening.
- Samples should be taken in an environment where the moisture content of the seeds, both to use for distribution and to return to storage, will be minimally affected.
- Samples should be shipped in airtight containers to ensure that the moisture content of the seeds does not change during transit.

Figure 9. Summary diagram of the workflow and activities for distribution of germplasm

Distribution of germplasm	
Genebank complies with national, regional and international regulations and agreements	- Use Standard Material Transfer Agreement if signatory to Treaty and Annex 1 material - If SMTA is not applicable, negotiate a Material Transfer Agreement with recipient (SMTA can also be used)
A policy is in place for the number of seeds to distribute for any given species	- Regenerate accessions with too few seeds
Required documentation is requested and obtained	- Request import permit regulations from the relevant national authority of the receiving country
Arrangement with National Plant Protection Officers is made for germplasm inspection and issuance of phytosanitary certificate	
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
Required documentation is placed both inside and outside of shipping package	- Include accessions 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
Packaging material and transport allows for safe and timely delivery	- Use of packaging and shipping guidelines/recommendations similar to those utilized for acquisition
Status of germplasm and condition on arrival is obtained	- Track the shipment and follow up with the recipient
Record, validate and upload all distribution and exchange data, including associated metadata	

9. Personnel and security

9.1 Personnel

It is recommended that the genebank have a strategy in place for personnel, including a succession plan; a corresponding budget must be allocated and reviewed regularly.[51]

✓ **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 a minimum of well-trained staff with clearly defined responsibilities for accession management.[52] 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;
- 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; and
- retaining competent staff by providing recognition and rewards for excellent performance.

✓ **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.

9.2 Security

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.[53] This strategy and an accompanying action plan should be regularly reviewed and updated to take changing circumstances and new technologies into account.

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

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

- 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.

[51] See Figure 10 at the end of this section for a summary diagram of the workflow and activities for personnel and security.
[52] Standard 6.8.3.
[53] Standard 6.8.1.

- 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 deal with those risks for which the current total risk rating is considered unacceptable, giving top priority to the highest assessed residual risks.
- Monitoring and review: analyse the risk management system and assess whether changes to the system are needed. Responsibilities for monitoring and review should be clearly defined and documented.

✓ **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.[54] 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.**

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 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.

[54] Standard 6.8.2.

Figure 10. Summary diagram of the workflow and activities for personnel and security

<table>
<tr><td colspan="2">Personnel and security</td></tr>
<tr><td>Human resources plan and appropriate annual budget allocation in place</td><td>- 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</td></tr>
<tr><td>Risks associated with staffing are included in the risk identification, analysis and management</td><td></td></tr>
<tr><td rowspan="7">Risk management strategy in place</td><td>Communication and consultation</td></tr>
<tr><td>Establishing the context</td></tr>
<tr><td>Risk identification</td></tr>
<tr><td>Risk analysis</td></tr>
<tr><td>Risk evaluation</td></tr>
<tr><td>Risk treatment</td></tr>
<tr><td>Monitoring and review</td></tr>
<tr><td>Staff member(s) appointed and trained in overseeing occupational safety and health</td><td>- Ensure all staff are aware and trained in occupational safety and health</td></tr>
<tr><td rowspan="2">Individual risks managed</td><td>Risks to Staff
- Take health and safety of staff and environment into consideration when applying pesticides
- Choose appropriate and approved agrochemicals
- Provide protective equipment and clothing and ensure its use</td></tr>
<tr><td>Risk to Collection
- Develop a risk management plan that includes mitigation and response contingencies for all potential risks to the physical collection</td></tr>
</table>

10. Infrastructure and equipment

This section considers the suggested infrastructure and equipment for handling recalcitrant and intermediate seeds in a genebank (Table 3). Genebanks handling non-orthodox seeds are generally equipped with: (a) basic viability testing equipment, growth rooms or incubators, and support facilities; (b) equipment to determine and reduce seed moisture content; (c) microscopes and analytical and molecular equipment for germplasm authentication and performance and stability testing; and (d) safety equipment, such as alarms and smoke detectors. If tests of seed storage behaviour are to be performed, equipment for measuring seed moisture content and for controlled drying are required. For seed moisture content, there should be an oven that can maintain 103°C for 17 hours, crucibles, a desiccator and balance. To dry seeds to intermediate levels, it may be necessary to remove seeds from a drying room or other drying environment after relatively short periods of time. Alternatively, seeds can be equilibrated at an intermediate relative humidity, in a humidity-controlled growth / test chamber or over an appropriate salt solution (Hay *et al*., 2023).

Factors that should be considered if designing or modifying genebank facilities include: (a) function of the facility (active collections, research and long-term storage);

(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 staff.

References are available for conserving species with recalcitrant or intermediate seeds, and these are included in the Further Information/Reading section. 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.

Table 3. General infrastructure and equipment recommended for a handling recalcitrant seeds in a genebank

Genebank operation/management area
General needs
Office space and supplies; computers, printers and accessories; 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.
Temporary storage of non-orthodox seeds and testing for storage behaviour.
Oven, analytical balance (accuracy to 0.001 mg), crucibles and desiccators with silica gel or other desiccant for moisture content determination.
Drying room and associated seed processing room and/or humidity cabinets or air-tight containers and salt solutions to equilibrate seeds to different moisture levels.
Automated data loggers to monitor temperature and humidity at regular intervals when storing non-orthodox seeds.
Sterile plastic buckets or boxes with a mesh platform or polyethylene bags for short-term storage.
Incubators/refrigerators or cold room(s) (6 and/or 16°C), with shelving system, thermostat, temperature recording, low- and high- temperature alarm. Cold rooms should have personnel panic button.
Seed viability monitoring.
Germination test facilities including media preparation area, test set-up/scoring area, dissection equipment, microscopes, controlled environment facility (plant growth room, germination chamber(s), incubator(s)), viability test sheets, data sheets or mobile devices for electronic data recording, barcode reader.
Preparation of material for long-term conservation.
See FAO (2022b; table 2) for field collections, FAO (2022c; table 2) for in vitro culture, and FAO (2025) for cryopreservation.
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 seed-lot inventory and viability, and flagging accessions requiring regeneration. Data backup/storage
Distribution
Balances, seed counter, tri-laminate foil bags, bag sealer, labels (preferably barcoded), packing materials. Data sheets or mobile devices for electronic data recording, barcode reader.
Security and personnel
Generator(s), fire-extinguishing equipment, security cameras, alarm systems, security doors. Protective clothing and protective gear such as dust masks, gloves and footwear.

11. References

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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 collected sample	Develop and follow an agreed collecting strategy and methodology that adequately follow genetic sampling guidelines
Taxonomic misidentification	Include a taxonomist in the collecting team and have genebank staff trained in taxonomy Take herbarium vouchers and photos for verification by experts Ensure that data-collection sheets include other descriptors to be recorded during the collecting mission
Mislabelling/loss of labels	Firmly attach one label to the outside of each collecting bag; place another label inside the collecting bag
Transcription errors	Consider the use of mobile devices, ensuring regular data backup and availability of sufficient charged batteries Implement data validation
Loss of viability during collecting missions/transport leading to reduced seed longevity (and earlier regeneration)	Ensure timely transfer to controlled drying conditions Ensure appropriate post-harvest handling according to the maturity of the seeds and the prevailing environmental conditions

Storage of non-orthodox seed

Risk	Risk control/mitigation
Reduced seed longevity due to unwanted moisture loss or uptake during storage	Pack seeds in a controlled environment Set up a monitoring system to periodically measure the moisture content of randomly selected samples from the genebank and of any accessions removed for testing or distribution If storage system should be air-tight (intermediate seeds), leak-test packaging material and ensure seals are air-tight.

Contamination with pathogenic micro-organisms	Work in a clean and sterile environment wherever possible
Mixing/mislabelling of samples	Pack carefully to avoid mixing Place labels inside and outside of packets Use computer-generated barcode labels to minimize errors
Stored samples fall below viability or quantity thresholds	Ensure that the documentation system includes automated tools for monitoring seed-lot viability and inventory and flag accessions requiring regeneration
Inadequate storage temperature due to power failure	Ensure backup generators and fuel are available

Seed viability monitoring and testing for non-orthodox behaviour

Risk	**Risk control/mitigation**
True viability of accessions is not reflected during germination testing	Optimize germination testing and dormancy breaking methods. Use replicated testing procedures Carry out cut tests to identify seeds that are still firm/fresh to estimate the viability of dormant accessions Outsource germination testing if necessary
Inappropriate viability testing intervals result in depletion of seeds or significant fall in viability	Use all available viability-monitoring data (for example, germination rate, and number of abnormal seedlings) for the accession and collection to set appropriate monitoring intervals. Consider shortening the monitoring intervals when seed lots are known/predicted to be approaching the viability threshold.

Preparation of non-orthodox material for long-term conservation

Risk	**Risk control/mitigation**
Loss of genetic diversity and or viability of accessions	Seed are used as soon as possible upon arrival at genebank Optimize all protocols used A sufficient number of propagules are conserved to capture genetic diversity and safety of each accession
Mixing/mislabelling of samples	Verify accession labels during all stages Use computer-generated barcode labels to minimize errors

Distribution

Risk	**Risk control/mitigation**
Mixing/mislabelling of samples	Pack carefully to avoid mixing Use labels on the inside and the outside of seed packets Use computer-generated barcode labels to minimize errors
Viability loss due to delayed or damaged shipments	Pack seeds in suitable packaging to minimize uptake of moisture. Ensure seeds are dispatched promptly, and use the fastest and safest way of sending them

Appendix: Examples of economically important species producing non-orthodox seeds

Species		Common name	References
Recalcitrant			
Artocarpus heterophyllus Lam.	Moraceae	Jackfruit	Grabe (1989)
Artocarpus integer (Thunb.) Merr.	Moraceae	Cempedak	Hor *et al*. (1990)
Avicennia marina (Forssk.) Vierh.	Acanthaceae	Mangrove	Farrant *et al.* (1986)
Camellia sinensis (L.) Kuntze	Theaceae	Tea	Grabe (1989)
Cocos nucifera L.	Arecaceae	Coconut	Engelmann (1999)
Dimocarpus longan Lour.	Sapindaceae	Longan	Chin *et al*. (1984)
Durio zibethinus L.	Malvaceae	Durian	Hor *et al*. (1990); Grabe (1989)
Elettaria cardamomum (L.) Maton	Zingiberaceae	Cardamom	Grabe (1989)
Euterpe oleraceae (Mart.)	Arecaceae	Acai palm	Panza *et al.* (2007)
Hevea brasiliensis (Willd. Ex A.Juss.) Müll.Arg.	Euphorbiaceae	Rubber	Chin *et al.* (1981)
Litchi chinensis Sonn.	Sapindaceae	Lychee	Ray and Sharma (1987)
Mangifera indica L.	Anacardiaceae	Mango	Tang *et al*. (2008)
Nephelium lappaceum L.	Sapindaceae	Rambutan	Hor *et al.* (1990)
Persea americana Mill.	Lauraceae	Avocado	Raja *et al.* (1991)
Sicyos edulis Jacq.	Cucurbitaceae	Chayote	Ellis (1991)
Theobroma cacao L.	Malvaceae	Cocoa	Hor *et al*. (1984)
Intermediate			
Citrus spp.	Rutaceae	Various	Hong *et al*. (2001); Xue and Wen (2018); Damasco and Refuerzo (2016)
Coffea arabica L.	Rubiaceae	Coffee	Dussert *et al*. (2006)
Elaeis guineensis Jacq.	Arecaceae	Oil palm	Ellis *et al*. (2007)
Mimusops elengi L.	Sapotaceae	Spanish Cherry, Medlar	Mai-Hong *et al.* (2006)
Phoenix reclinata Jacq.	Arecaceae	Wild date palm	Von Fintel *et al.* (2007)

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