Food and Agriculture Organization of the United Nations

COMMISSION ON GENETIC RESOURCES FOR FOOD AND AGRICULTURE

Practical guide for the application of the Genebank Standards for Plant Genetic Resources for Food and Agriculture

Conservation via *in vitro* culture

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Foreword

The international development community and governments are striving to achieve the Sustainable Development Goals (SDGs), including the eradication of hunger, by 2030. The imperative of generating and disseminating the solutions that work for farmers as means to achieve the SDGs provides the backdrop for FAO's Strategic Framework 2022-2031. The Strategic Framework aims to transform current sub-optimal agricultural and food systems to become more efficient, inclusive, resilient and sustainable as envisaged in its four aspirations: better production, better nutrition, a better environment and a better life.

With about 80 percent of food being plant-based, these efforts will benefit greatly from sustainable crop production systems, which generate increased yields of nutritious food with fewer external inputs than are currently the case, even under worsening climate change scenarios. One critical element of such systems is a diverse suite of progressively superior crop varieties that are input use-efficient, nutritious, adapted to target agroecologies, and resilient to biotic and abiotic stresses. Plant breeders need access to the widest possible spectrum of the sources of heritable variations in order to breed such crop varieties. Plant genetic resources for food and agriculture (PGRFA) – which include improved crop varieties, farmers' varieties/landraces and the wild relatives of crops – are the sources of such variations. The safeguarding of characterized and documented PGRFA in genebanks is a reliable means to ensure their availability to current and future generations – both for direct use and for research and plant breeding.

FAO and partners have been cognizant of the critical importance of effective genebank operations to sustainable crop production systems. In addition, in recognition of the global interdependence on PGRFA, facilitated through the exchange of germplasm, the need for the harmonization of genebank procedures has always been at the forefront of FAO's work on the conservation and sustainable use of PGRFA. This was why FAO, through

its Commission on Genetic Resources for Food and Agriculture, published the *Genebank Standards for Plant Genetic Resources for Food and Agriculture* (Genebank Standards) in 2014. The Genebank Standards provide international standards for *ex situ* conservation of PGRFA in seed genebanks, field genebanks, *in vitro* culture and cryopreservation.

Deemed a seminal reference material, one of the feedbacks provided by genebank practitioners was that the utility of the Genebank Standards would be enhanced through the development of companion volumes that detail the action steps of the genebank workflow in a sequential manner and provide guidance on the complex steps and decisions required. In response to this feedback, FAO developed the *Practical guide for the application of the Genebank Standards for Plant Genetic Resources for Food and Agriculture: Conservation via* in vitro *culture.* In addition, the *Practical guide for the application of the Genebank Standards for Plant Genetic Resources for Food and Agriculture: Conservation of orthodox seeds in seed genebanks* and the *Practical guide for the application of the Genebank Standards for Plant Genetic Resources for Food and Agriculture: Conservation in field genebanks* have also been developed.

These companion volumes, prepared in an easy to understand format, will be useful for genebank technicians as operational handbooks; for genebank managers as streamlined instructional materials and for all interested in genebank operations, a handy reference material.

Jingyuan Xia
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Preface

The *ex situ* conservation of plant genetic resources for food and agriculture (PGRFA) in genebanks is aimed at safeguarding them for use by current and future generations – both directly by end users and as materials for research and plant breeding. Genebanks, therefore, ultimately contribute to sustainable crop production systems and hence, food security and nutrition. However, genebanks must be managed effectively in order to conserve these resources in optimal conditions and making them available for use.

Genebanks also play a major role in fostering global collaboration on PGRFA through germplasm exchange, including across national boundaries. The *Genebank Standards for Plant Genetic Resources for Food and Agriculture* (Genebank Standards), published in 2014, aimed at the harmonization of genebank operations, i.e. the storage of the accessions, their characterization and evaluation and the documentation of associated data, across genebanks and countries. The Genebank Standards set the benchmark for current scientific and technical best practices.

Addressing an identified need for the articulation of the stepwise activities of routine genebank operational workflows, the *Practical guide for the application of the Genebank Standards for Plant Genetic Resources: Conservation via* in vitro *culture* was developed. Endorsed by FAO's Commission on Genetic Resources for Food and Agriculture at its Eighteenth Regular Session in 2021, this Practical Guide presents the information contained in the Genebank Standards in a format that presents the action steps of genebank workflow in a sequential manner. The series of interrelated operations presented, are based on the underlying principles of genebank management, namely: identification of accessions, maintenance of viability, maintenance of genetic integrity during storage and regeneration; maintenance of germplasm health; physical security of collections; availability, distribution and use of germplasm; availability of information; and proactive management.

The sections included in this practical guide are: acquisition of germplasm; *in vitro* culture and slow-growth storage; recycling and rejuvenation; characterization and evaluation; documentation; distribution; safety duplication; and security and personnel. A summary diagram of the associated workflow and activities supports each of these operations. An additional section considers the suggested infrastructure and equipment for designing or modifying the facilities for *in vitro* genebanks. A final section provides a list of references to provide guidance and/or technical background on *in vitro* genebank operations and management. An annex identifies the potential risks associated with the different genebank operations and their respective proposed preventive measures.

This practical guide is part of a series of publications conceived as companion volumes to the Genebank Standards aimed at facilitating their more widespread application. Genebank managers may use the practical guide as a basis for the development of standard operating procedures, quality management systems or, simply, as a handbook.

1. Introduction

***In vitro* sweetpotato, CIP**

Many field and horticultural crops as well as agroforestry species are difficult or impossible to preserve as seeds. These include: species that only produce recalcitrant seeds with a short lifespan in seed storage; species for which seed production may take many years, as is the case for many tree species: species that are heterozygous and therefore do not produce true-to-type seeds; and species that do not produce seed at all and are vegetatively propagated. Other examples include males of dioecious species and rare plants that are under threat of overgrazing and for which time to produce seeds before the population totally vanishes is limited. *In vitro* conservation offers an option for these species. Additionally, *in vitro* techniques provide a germplasm storage procedure that combines the possibility of disease elimination with that of rapid clonal propagation, thus providing a means by which germplasm can be safely exchanged and distributed.

In vitro slow growth storage techniques are being routinely used for medium-term conservation of numerous species of both temperate and tropical origin, including crop plants (e.g. potato, yam and cassava), and rare and endangered species. Germplasm can be stored for between several months and 2–3 years without subculture, depending on the technique used and the genotype of the plant material.

In vitro genebanks are underpinned by the same principles as other genebanks, namely identification of accessions, maintenance of viability, maintenance of genetic integrity during storage and regeneration, maintenance of germplasm health, physical security of collections, availability, distribution and use of germplasm, availability of information and proactive management (FAO, 2014: Chapter 2).

Conservation in genebanks by means of *in vitro* culture can be broken down into a series of interrelated operations (Figure 1). This practical guide for conservation in genebanks

by means of *in vitro* culture presents practices and activities[1] critical to each operational area (Table 1). It outlines workflows for routine genebank operations for conservation via *in vitro* culture (Figure 2), and supports the application of the *Genebank Standards for Plant Genetic Resources for Food and Agriculture* (Genebank Standards) (FAO, 2014).[2] The purpose of this guide is to present the information contained in the Genebank Standards in a format that details the actions of the genebank workflow in a sequential manner and thereby facilitates more widespread adoption of the Genebank Standards. Genebanks may use the activities outlined in this guide as a basis for the development of standard operating procedures (SOPs) (IITA, 2012) and quality management systems (QMS) (CGIAR Genebank Platform, 2021) for conserving these 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 operating a genebank for *in vitro* culture. Each genebank will have its own circumstances, and the efficient management of particular collections will require careful consideration and procedural adjustments based on experience. For detailed technical specifications of the steps outlined in this guide, genebank staff will need to consult various sources of information, a few of which are referenced in this booklet.

Figure 1. Major operations for conservation via *in vitro* culture

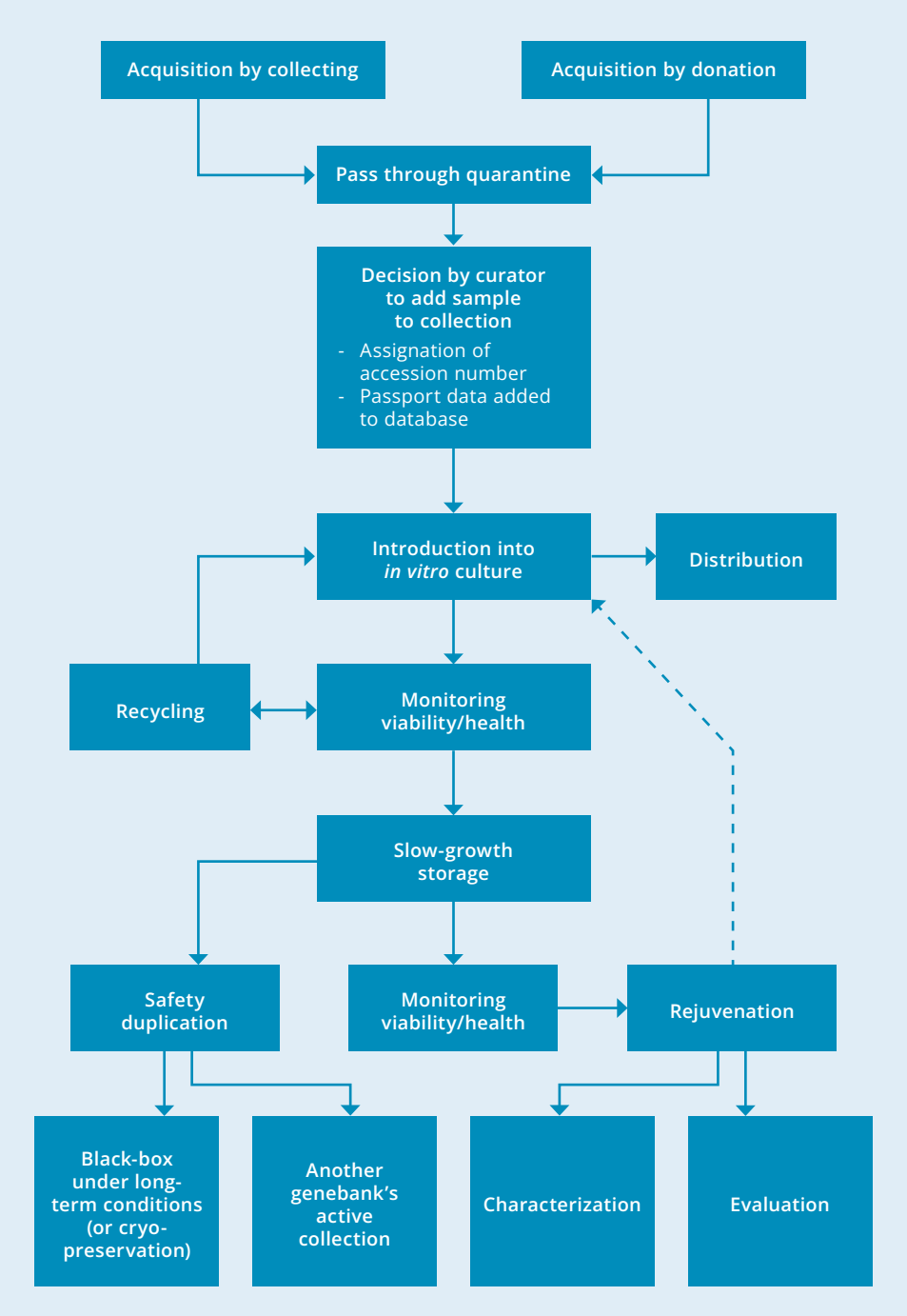
1 Practices and activities follow best practices as outlined in the Genebank Standards.

2 All standards referenced throughout the document are described in the FAO Genebank Standards.

Table 1: Underlying principles and related genebank operations for *in vitro* genebanks

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 through genebank operations and in the laboratory, field and greenhouse/screenhouse
Maintenance of viability	Best practices followed and timing optimized during collection, processing, introduction into *in vitro* culture and slow-growth storage, regeneration and transportation *In vitro* culture and slow-growth storage conditions optimized and monitored Germplasm health monitored regularly Recycling/rejuvenation undertaken when necessary
Maintenance of genetic integrity	Collection and maintenance of samples conducted in a manner that ensures they represent the original population as much as possible Best practices followed during packaging, introduction into *in vitro* culture and slow-growth storage and recycling/rejuvenation Genetic stability evaluated
Maintenance of germplasm health	Quarantine procedures undertaken when needed Best practices followed during collection, processing, introduction into *in vitro* culture and slow-growth storage, regeneration and transportation Contamination monitored and managed in the laboratory and in the field or greenhouse
Physical security of collections	Risk management strategy developed and implemented Appropriate genebank infrastructure in place and maintained Accessions safety duplicated/safety backed up
Availability and use of germplasm	Germplasm acquired and distributed according to legal and phytosanitary requirements Sufficient inventory and efficient and timely dispatch of samples ensured 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 in a genebank for *in vitro* conservation.
Note: Each step is associated with proper documentation.



2. Acquisition of germplasm

Collecting coconut, Indonesia

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

✔ **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.

✔ **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, the International Treaty on Plant Genetic Resources for Food and Agriculture (Treaty) or the Convention on Biological Diversity (CBD) for genetic resources access.

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

[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.

✔ **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 the associated data 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 associations 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 in order to prevent duplicates and of having a clear strategy for collecting missions that considers 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

[5] Standard 6.1.2.

- plan the mission well in advance 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:

- 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 *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.[7]**

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 samples through the relevant quarantine process before they are transferred to the genebank, if required; and
- processing collected materials in containment or in an isolated area, according to the advice of the national phytosanitary authority.

6 Standard 6.1.1.

7 Standard 6.1.1.

✔ **Collecting missions are scheduled at the optimum stage of maturity/growth and propagules are collected from visibly healthy plants, devoid of disease and insect pest infestations or other damage.[8]**

It may be necessary to engage a local expert if the species is not known to genebank staff in order to ensure the quality and viability of the collected sample, whether vegetative or recalcitrant seeds (or their fruits). Collecting late-season recalcitrant seeds of any species should be avoided. Whole fruits of uniform maturity status should be collected from the parent plants prior, but as close as possible, to natural abscission. Avoid collecting fallen fruits from the ground, especially those showing damage or signs of weathering. Seasonality is a consideration for the collecting of bulbs, tubers and woody species.

✔ **Propagules/explants are collected from an appropriate number of individual plants,[9] but the depletion of the natural population targeted for collecting is avoided.**

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 type and size of the propagule (see SGRP-CGIAR, 2011).

- It is recommended to harvest from at least 30 individuals for cross-fertilizing species and 60 individuals for autogamous species, if possible.
  - If collecting recalcitrant seeds, 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 target population.
  - For roots and tubers, collect a minimum of four propagules for each sample, more if culturing techniques for that species are not reliable (Dansi, 2011).
  - If collecting woody stems, increase sample size to allow for any problems (and therefore losses) in decontamination. Approximately 5–10 cuttings/propagules per plant has been recommended (see Thompson, 1995).
- Note: Collecting *in vitro* materials offers an alternative for germplasm collection and transport, and is particularly useful for species that are vegetatively propagated and for those with recalcitrant seeds or embryos, which deteriorate rapidly. However, transportation times will still have to be minimized.
  - Explants collected *in vitro* are often surface-decontaminated using 70 percent ethanol, followed by NaOCl or commercial bleach that generally contains about 3 percent active chlorine. Alternative sterilants, such as dilute solutions of 0.5–2 percent (weight/volume) calcium hypochlorite, may also be used. After decontamination, the explant is usually trimmed to a final size for transport, including removal of the dead zones caused by the sterilizing solution penetrating the cut surfaces. See Pence and Engelmann (2011) for additional guidance on *in vitro* collecting techniques.

[8] Standard 6.1.3.

[9] Standard 6.1.3.

✔ **Collected samples are labelled and are not mixed during handling.**
Use indelible ink or computer-generated labels (preferably with barcodes), if possible, on the sample packet to label the sample. Placing labels both inside and outside a seed packet is a good practice. Protecting inside labels from deterioration is useful if the seed/plant material is not dry. It is recommended to keep a journal with all collection numbers assigned to each samples and additional information, as required.

✔ **Collected germplasm is accompanied by the associated data outlined in the FAO/Bioversity Multi-Crop Passport Descriptors.[10]**
A standardized collecting form is helpful for collecting the associated data for each sample obtained. Each sample is assigned a collection number so the samples can be linked to the collected information. Collecting the following information may be considered:

- Taxonomic identification at species and intraspecific levels if possible, 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.
- 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 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:

- Collection number
- Collecting institute name/code
- Taxon name, as detailed/specific as possible
- Common crop name
- Location of collecting site
- Latitude of collecting site
- Longitude of collecting site
- Elevation of collecting site
- Date of collecting
- Biological status (wild, weedy, landrace, etc.)

[10] Standard 6.1.2.

✔ **The period between collecting and processing and then transferring to the genebank is as short as possible to prevent loss and deterioration of the material.**[11] Recalcitrant seeds are sensitive to desiccation and chilling injury. Water loss curtails storage life span. Similarly, clonal stocks do not retain viability for a long period of time and vegetative propagules decay easily and quite fast. Transport in tropical countries, where high temperatures and humidity prevail and where transport may be difficult, slow and uncertain, can be the most challenging. Under such conditions, special care must be taken to ensure that samples are not left in the sun and are stored under shade at all times.

✔ **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) should be taken into account in order to ensure that the material reaches the destination genebank in good condition. The following considerations may decrease the risk of germplasm loss after collecting missions:

Packaging

- Precautions should be taken to avoid risk of fungal or insect attacks during shipment.
  - If a pest has been observed and correctly identified, it may be necessary to apply pesticide before packaging. Avoid any unnecessary chemical treatment, as it may be harmful to the collected samples.[12] If applied, declare treatments on each package and in accompanying documentation.
- For recalcitrant seeds, it is important that water content be maintained upon collecting and during transport by maintaining high relative humidity (RH) in the storage containers.
  - Where possible, recalcitrant seeds are best transported within the fruits, both for protection and to avoid dehydration.
  - For species with very large fruits or fruits that can be easily damaged during transport, extracting seeds and surface disinfection before packaging should minimize fungal proliferation.
- Scions are best packed in sterile cotton or other suitable material in a perforated plastic bag to ensure sufficient air exchange.
- Rigid cushioned envelopes or insulated packaging should protect samples from crushing by mechanical mail sorters and deterioration (in the case of fleshy fruits).
- If available, *in vitro* plantlets are a safe way of moving germplasm. *In vitro* collected samples should be placed in sterile transparent watertight sealable plastic vials and packed firmly, but not too tightly, in a box or carton, with addition of crumpled paper or polystyrene material to protect against shocks.

[11] Standard 6.1.4.

[12] Many of the fruits of plants with recalcitrant seeds are contaminated with fungi, even when they are not visible. Surface disinfection must therefore be carried out prior to transport.

Transport

- For long transit times by road, periodic aeration of the collected material may be necessary as a precaution against viability lost.
- Sending shipments by the fastest means possible, either by airfreight or by courier, should avoid deterioration of sample quality and long exposure to adverse environmental conditions.
- Continuous tracking of the package, if possible, will ensure genebank staff are prepared to process the samples upon their arrival at the genebank.
- Note that for some crops, such as *Musa* and cocoa, shipment of material through transit or quarantine centres in non-producing third countries may be the best solution.

✔ **All incoming material is checked for damage/contamination in a designated reception area (e.g. plant health unit) and processed in a way that does not alter the physiological status.[13]**

- Low-quality or contaminated plant materials are not planted directly in the field.
- Decontamination activities, such as treating samples with a surface disinfectant agent, are used to remove all adherent microorganisms, taking into account any decontamination treatment given prior to packaging and transport.
- Quarantine measures are applied as necessary.

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 a 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, a Material Transfer Agreement (MTA) is usually used (e.g. AVRDC, 2012), though a SMTA could also be used.
- 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 6.1.5.

[14] Standard 6.1.1.

✔ **Donated germplasm is accompanied by the associated data outlined in the FAO/Bioversity Multi-Crop Passport Descriptors.[15]**
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).

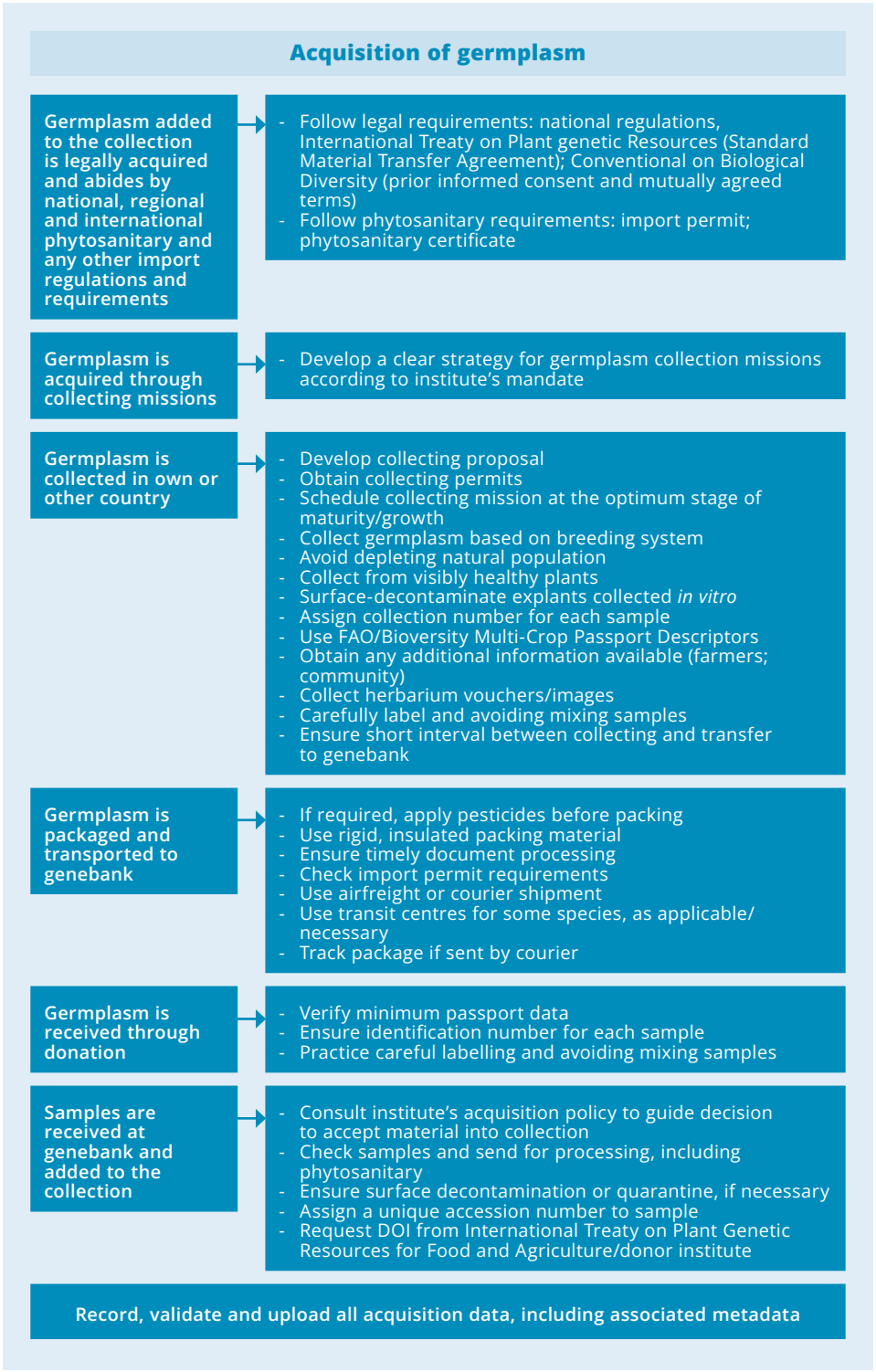
✔ **The genebank abides by national, regional and international phytosanitary and any other import regulations and requirements from the relevant authorities.**
When germplasm is moved there is a risk of accidentally introducing plant pests and diseases along with the host plant material. The following 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 process before they are transferred to the genebank, if required; and
- processing donated materials in containment or in an isolated area, according to the advice of the national phytosanitary authority.

✔ **All incoming material is checked for damage/contamination in a designated reception area (e.g. plant health unit) and processed in a way that does not alter the physiological status.[16]**
- Low-quality or contaminated plant materials are not planted directly in the field.
- Decontamination activities such as treating samples with a surface disinfectant agent are used to remove all adherent micro-organisms, taking into account any decontamination treatment given prior to packaging and transport.
- Quarantine measures are applied as necessary.

[15] Standard 6.1.2.

[16] Standard 6.1.5.

Figure 3. Summary diagram of the workflow and activities for acquisition of germplasm



3. *In vitro* culture and slow-growth storage

©CIAT/Georgina Smith

Plant Resources Center Genebank, Viet Nam

The genebank should have a documented policy and/or procedure, as applicable, for *in vitro* culture and slow-growth storage, including guidelines and methodologies for explant identification, initiation into *in vitro* culture, recycling/rejuvenation, media composition, and both light and temperature regimes.[17]

A. *In vitro* culture

✔ **The culture media composition for initiating the explant *in vitro* and for multiplication is determined according to the species.**
It may be necessary to carry out a literature review to investigate whether conditions for *in vitro* culture have been established for the target genotype or any related species. In most cases, modifications to published techniques will be required or new techniques developed for taxa not cited in the literature.

✔ **The appropriate type of explant and the optimum time (growth stage and physiological age of parent plant) for initiation into culture are determined for a particular genus or species from the literature or by experimentation.**
There are various types of explants frequently used for initiation into culture: nodal segments, apical meristems, roots, cotyledons, embryos, leaf discs, leaf blades, pedicles, petioles, anther, ovaries, etc.

✔ **Explants are free from known diseases and microbial contaminants.**
To ensure viable and disease-free establishment, the following practices should be considered:
- obtaining explants from vigorous and healthy mother plants;

[17] See Figure 4 at the end of this section for a summary diagram of the workflow and activities for *in vitro* culture and slow-growth storage.

- indexing mother plants to determine the presence/absence of known viruses:
  - routine indexing procedures include enzyme-linked immune-sorbent assay (ELISA), polymerase chain reaction (PCR), reverse transcriptase PCR (RT-PCR) and non-radioactive probe-based nucleic acid spot hybridization (NASH) techniques were developed and validated for routine testing (Selvarajan *et al*., 2009);
- applying surface decontamination methods to eliminate contaminants from explants excised from field-grown or greenhouse-grown material (*ex vivo*):
  - examples include sterilizing using bleach solution, hot water treatment and treatment with ozone dissolved in water (Umber *et al*., 2020);
- transferring explants to a rich detection medium, which favours micro-organism growth and therefore allows for early determination of contamination, and treatment or elimination of contaminated cultures (see Reed and Tanprasert 1995; Reed *et al*., 2004); and
- if necessary, regenerating *in vitro* plantlets from virus-infected plants and using various chemical or thermotherapy techniques to produce virus-free material before long-term conservation.

✔ **Once successfully initiated into culture, the accession is multiplied for either normal growth (active growing conditions) or slow growth storage.**
In vitro cultures serve as sources of disease-free materials for distribution and multiplication and as a source of explants for cryopreservation. Regular monitoring, and safe removal and disposal of infected materials, is essential.

Rapid propagation of selected materials is necessary for research or distribution. It is important to note that the multiplication rate strongly depends on the genotype of the accession and is influenced by the composition of the medium (particularly the cytokinin concentration), the explant size, age of culture and the size of the culture vial (SGRP-CGIAR, 2010a).

✔ **Any cultures exhibiting somaclonal variation are discarded.**
Somaclonal variation is the result genetic or epigenetic changes that arise *in vitro* among clonal regenerates and their corresponding donor plants (see Leva and Rinaldi, 2017). The occurrence of somaclonal variation during *in vitro* culture has a negative effect on the rapid production of clonal plants for distribution and cultures in which this has happened must be discarded.

✔ **Culture containers are clearly labelled following genebank practice.**
Information on labels could include accession number, date of introduction and line number (number of cuttings from the accession).

B. Slow-growth storage

✔ **Slow-growth storage conditions are optimized for the target species.**[18]

It may be necessary to carry out a literature review to investigate whether conditions for slow-growth storage conditions have been established for the target species or genotype, or any related species. If this information is not available, then conditions will have to be established by experiment. Standard protocols have been published and can be used for guidance.[19] Slow-growth storage conditions can include:

- Physical growth limitation, including: (a) low temperature; (b) low light/restricted photoperiod; (c) minimal containment; (d) minimal O_2; and (e) osmotic (water) stress.
- Chemical growth limitation, including: (a) growth regulator retardation and (b) growth inhibitors.
- Nutrient limitation, including: (a) low macronutrient levels and (b) low micronutrients levels.
- Avoidance of the formation of callus and other abnormalities, such as hyperhydration, and somaclonal variation.
  - Material for *in vitro* conservation maintained as whole plantlets or shoots[20] can avoid hyperhydricity.
  - Techniques for avoiding hyperhydration include culturing on a medium containing 6-bensyladenine (BA), kinetin (Kin) or thidiazuron (TDZ) (Badr-Elden *et al*., 2012), and modifying the ratio of $NH4^+$/NO3 (see Ivanova and Van Staden, 2009; El-Dawayati and Zayed, 2017).
  - Avoiding the excess use of growth regulators in media can reduce the possibility of later callus formation in storage and thus minimize the risk of somaclonal variation. Avoiding too many subcultures can also decrease the risk of somaclonal variation.

✔ **Germplasm for storage is selected from young cultures that have not been subject to too many subcultures in order to minimize the chance of selecting a variant plant.**

As the storage capacity of *in vitro* cultures strongly depends on the initial quality of the cultures, the following practices are encouraged:

- visually assessing the general performance of each culture using the following criteria prior to selection for slow growth storage: vigour and absence of fungal and bacterial contamination, chlorosis, blackening or tissue necrosis;
- discarding contaminated and low-quality cultures immediately; and
- propagating cultures onto a new medium if all cultures under evaluation have been found to be below standard because at least one of the above criteria is not met.

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18 Standard 6.4.1.

19 See further information/reading section.

20 Standard 6.4.2.

- ✔ **The optimum storage conditions are selected by visually assessing the general performance of each culture using the following criteria: vigour, fungal and bacterial contamination, chlorosis, blackening, tissue necrosis, hyperhydration and etiolation.**
  Optimum storage conditions are minimal growth conditions that prove to be acceptable for most genotypes. Not all accessions and genotypes will respond equally well to the applied conditions. For cold-tolerant species, storage conditions often range from 0 to 5 °C; the lowest temperatures tolerated by many tropical species often range from 15 to 20 °C.[21]

- ✔ **The number of replicates to put into storage is determined.**
  It is important to maintain a sufficient number of replicates per accession to ensure that genetic integrity is maintained,[22] taking into account: (a) cost; (b) potential risks (the greater the risks, the larger the sample size); (c) the duration between subculture periods and how the slow-growth conditions affect the propagation potential (number of shoots/nodes available for multiplication after storage); and (d) the purpose of the collection (active or base). If an accession only produces a few plants per subculture and is used for active distribution, more replicates will be required than if the accession is solely in a backup collection.

- ✔ **Culture containers are clearly labelled following genebank practice.**
  Information on labels could include accession number, date of introduction and line number (number of cuttings from the accession).

- ✔ **Regular monitoring is carried out to detect and remove those *in vitro* cultures that exhibit any variation from whole plantlets, including somaclonal variation, contamination, hyperhydration, etc.[23]**

- ✔ **All *in vitro* culture and slow-growth storage data, including associated metadata, are recorded, validated and uploaded to the genebank information management system.**
  Data to consider include: type of explant; explanting/culture initiation date; initiation/establishment medium; multiplication medium; rooting medium; slow-growth storage medium; number of replicates for slow-growth storage; performance indicators for *in vitro* culture and slow-growth storage; number of subcultures and duration of subculture period; and any specific growth characteristics, such as callus formation during storage and tendency to become hyperhydrated. Consider the use of electronic devices to avoid transcription errors and for ease of uploading.

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21 See Section 6.4 of the Genebank Standards.

22 This will vary depending on species.

23 Standard 6.4.3.

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.

Figure 4. Summary diagram of the workflow and activities for *in vitro* culture and slow-growth storage

4. Recycling and rejuvenation

©FAO/A. Noorani

Recycling germplasm stored *in vitro*,
Indian Institute of Horticultural Research

The genebank should have a documented policy and/or procedure, as applicable, for recycling and rejuvenation, including guidelines and methodologies for monitoring, subculturing, acclimatization and transfer to the field.[24]

✔ **Inventory and health of samples in *in vitro* culture and slow-growth storage are monitored regularly.**
The genebank information management system ideally includes automated tools for checking inventory and accession health, and flagging accessions requiring recycling and rejuvenation. It is also important to take practical considerations into account in order to avoid handling an overwhelming number of accessions.

Recycling:

✔ **Subculturing is carried out at the end of the storage cycle, when accessions show obvious signs of deterioration and/or when stock becomes low and there is a need for multiplication or safety duplication.**
Accessions should be regularly monitored for signs of necrosis. At the end of a storage cycle, new cultures are best placed for a short period under optimal conditions to encourage regrowth before the start of the next storage cycle. For security of collections, it is prudent to maintain a few viable and healthy cultures of the previous subculture cycle as "spare materials" until the newly subcultured set is healthy and growing.

[24] See Figure 5 at the end of this section for a summary diagram of the workflow and activities for recycling and rejuvenation.

✔ **Genetic stability is periodically assessed by means of visual assessment and transfer to the field for morphological observations or by using cytological or molecular techniques.**
It is important to develop a system for monitoring quality, viability, stability and contamination. Once the material has been in storage for a given time, quantitative and qualitative monitoring criteria should be used to assess the viability of an accession and to identify when it should be subcultured.

Rejuvenation:

✔ **Those cultures requiring rejuvenation (transfer of accessions to the greenhouse and field, followed by re-initiation into tissue culture) are determined.**
Cultures that are too old and have gone through too many cycles of recycling are rejuvenated. The timing of when rejuvenation is required will depend on the genotype and the *in vitro* conditions.
- Often, a threshold value is established based on experimentation (or is known from the literature). A threshold value is the number of cultures for a given genotype at which experiments have shown vigour declines and/or cultures become too old.
- If the number of cultures reaches this threshold, the accession should be transferred to the greenhouse or field for rejuvenation and re-initiation into tissue culture.

✔ **In the case of contamination of all replicates, material is subjected to rejuvenation and/or a decontamination treatment.**
Surface decontamination can be carried out using 70 percent ethanol, followed by NaOCl or commercial bleach that generally contains about 3 percent active chlorine. Alternative sterilants, such as dilute solutions of 0.5–2 percent (weight/volume) calcium hypochlorite, can also be used.

✔ **Selected germplasm undergoes an acclimatization process prior to transfer to the greenhouse or field.**
The progressive change of environment before the transfer to field conditions is called acclimatization or hardening, and includes first planting pots in a greenhouse environment. A number of practices are recommended, including:
- selecting plantlets showing well-developed root and shoot systems for acclimatization;
- removing any media from roots before planting in pots; and
- using sterile soil or planting medium.

✔ **Appropriate field management and cultural practices are applied.**

✔ **Optimal procedures are used to minimize risk to the genetic integrity of the accession.**

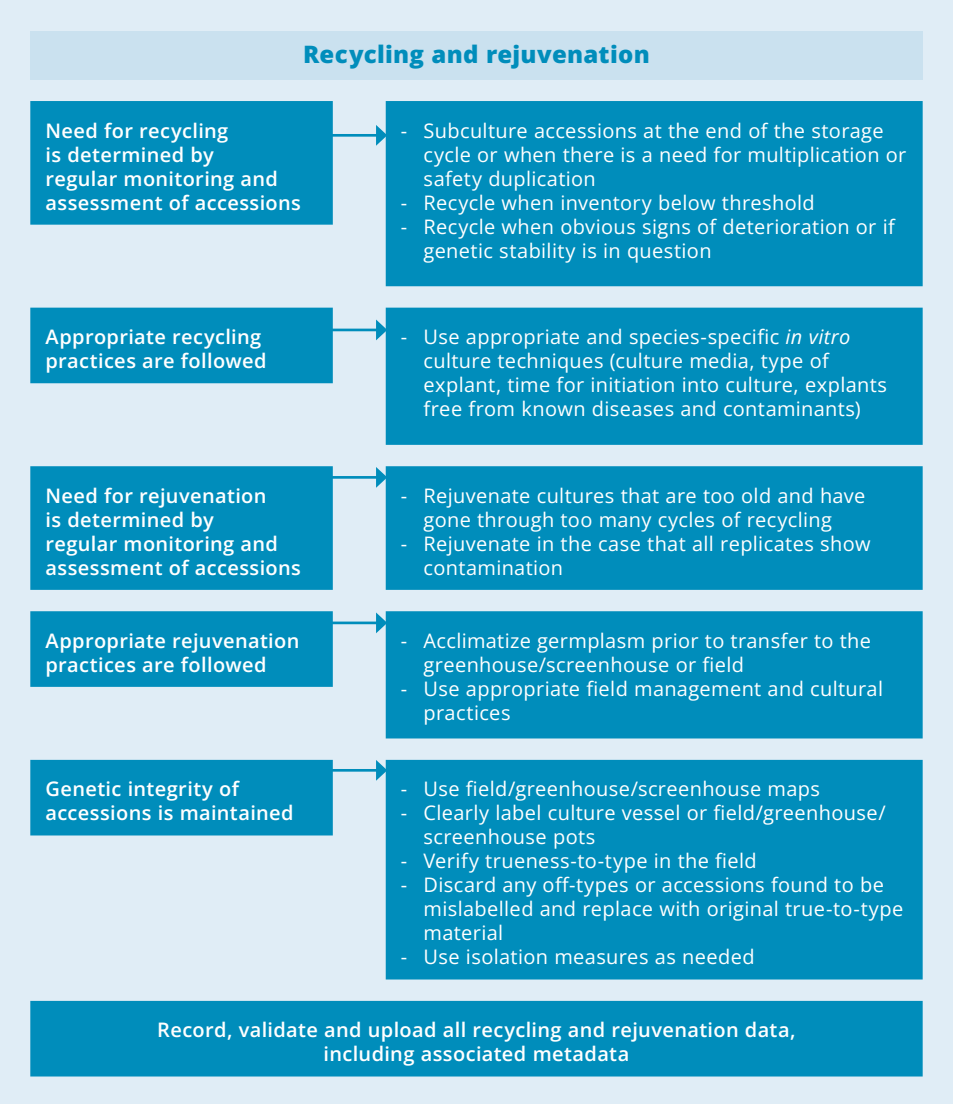
Accessions with the same characteristics as the original genotype are considered true-to-type. Assess trueness-to-type by comparing morphological and taxonomic characteristics of the plants with those of the original accession. Ideally, accessions are grown in a field collection next to the original mother plant.

- True-to-type accessions can be re-established in *in vitro* culture.
- Accessions identified as off-types with no value, or accessions that are found to be mislabelled, must be discarded and replaced with the original true-to-type material from the donor source.
- Note: Using field established plants to rejuvenate the accession in storage would require re-indexation for viruses, as the plants could have been exposed to them.

✔ **All recycling and rejuvenation data, including associated metadata, are recorded, validated and uploaded to the genebank information management system.**

Data to consider include inventory, date of subculture, date of initiating acclimatization, planting date, greenhouse and field cultural practices used, date of reinitiating into culture, 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 recycling and rejuvenation



5. Characterization and evaluation

Evaluation of pineapple germplasm *in vitro*, CePaCT Fiji

The genebank should have a documented policy and/or procedure, as applicable, for characterization and evaluation of germplasm, including step-by-step instructions describing sampling techniques, experimental designs, descriptors used (taxonomic, morphological, phenotypic, biochemical, nutritional, physiological and molecular) and the manner in which the data are collected and validated.[25]

- ✔ **Characterization and evaluation data are obtained for as many accessions as possible and as soon as possible.**
  Ideally, all accessions should be characterized and evaluated to maximize their utility. It is essential that staff be well trained in data recording, evaluation techniques carried out *in vitro* and field work.. In reality, genebanks are usually only able to evaluate subsets of their germplasm. It is therefore helpful to collaborate with national or international research organizations, with field stations in different agroecological environments, or with members of national or regional genetic resources networks. If germplasm is shared for evaluation purposes, it is recommended that a request be made for data to be sent back for inclusion in the genebank information management system.

- ✔ **Characterization and evaluation of most traits are carried out when accessions are taken out of *in vitro* conditions.**
  Taking accessions out of *in vitro* conditions provides an opportunity for characterization and evaluation data to be generated in the greenhouse or field. If germplasm is shared for evaluation purposes, it is recommended that a request be made for data to be sent back for inclusion in the genebank information management system.

[25] See Figure 6 at the end of this section for a summary diagram of the workflow and activities for characterization and evaluation.

✔ **Evaluation is carried out under *in vitro* conditions for certain easily screened traits, such as salt and drought tolerance.**
The correlation between evaluation data from *in vitro* and field conditions should be established first.

✔ **Germplasm is characterized for a set of highly heritable morphological traits, and species-specific characterization procedures are based upon standardized and calibrated measuring formats and categories, following internationally agreed descriptor lists as much as possible.**
The use of standardized crop descriptor lists and calibrated and standardized measuring formats enables the comparison of data across institutions and countries.[26] A wide range of crop descriptor lists has been developed, for example by Bioversity International (2018), the International Union for the Protection of New Varieties of Plants (UPOV, 2011), and the National Plant Germplasm System (NPGS) of the United States of America (USDA-ARS, 2021). If there are no existing descriptor lists for a species, it is recommended to use Bioversity International's Guidelines for Developing Crop Descriptor Lists (Bioversity International, 2007). It may be helpful to consider:
- using reference accessions in the same field to facilitate scoring;
- using herbarium specimens and possibly digital high-quality voucher images to guide true-to-type identification, including taxonomic identification and verification, if needed;
- observing and documenting the homogeneity/heterogeneity of an accession is important; and
- taking measurements at the plant level rather than at the plot level for crops with high levels of variability in order to capture information about the variability between plants of the same accession.

✔ **Experimental designs with replicates are used and evaluations conducted in different environments and/or over multiple years.[27]**
Traits measured during evaluation, such as yield and plant height, are mostly inherited through a large number of genes and therefore quantitative and subject to considerable environmental interaction. Consequently, they are more difficult to measure. Because of the strong genotype by environment (G x E) interactions, traits such as yield (and its components) are site-specific. Best practices to consider include:
- defining and identifying check accessions or varieties to be included in the statistical design and used over time, as they facilitate comparisons of data collected across locations and years;
- working with plant breeders and other specialists (for example, virologists, entomologists, mycologists, plant pathologists, chemists, molecular biologists

[26] See Standard 5.6.3.

[27] See Standard 5.7.3.

and statisticians) to agree on the traits to be evaluated, the accessions that will be tested and the experimental designs to be implemented;
- using appropriate screening protocols to make sure that internationally validated protocols are respected;
- creating both hard and electronic copies of field maps developed before planting; and
- clearly labelling plots (preferably with bar-codes).

✔ **Evaluation data are presented using appropriate methods.**
The use of standardized crop descriptor lists and calibrated and standardized measuring formats enables the comparison of data across institutions and countries. Data are either presented as discrete values (e.g. scores for severity of disease symptoms or symptoms of abiotic stresses) or as continuous values (e.g. length, height, weight) based on measurements.

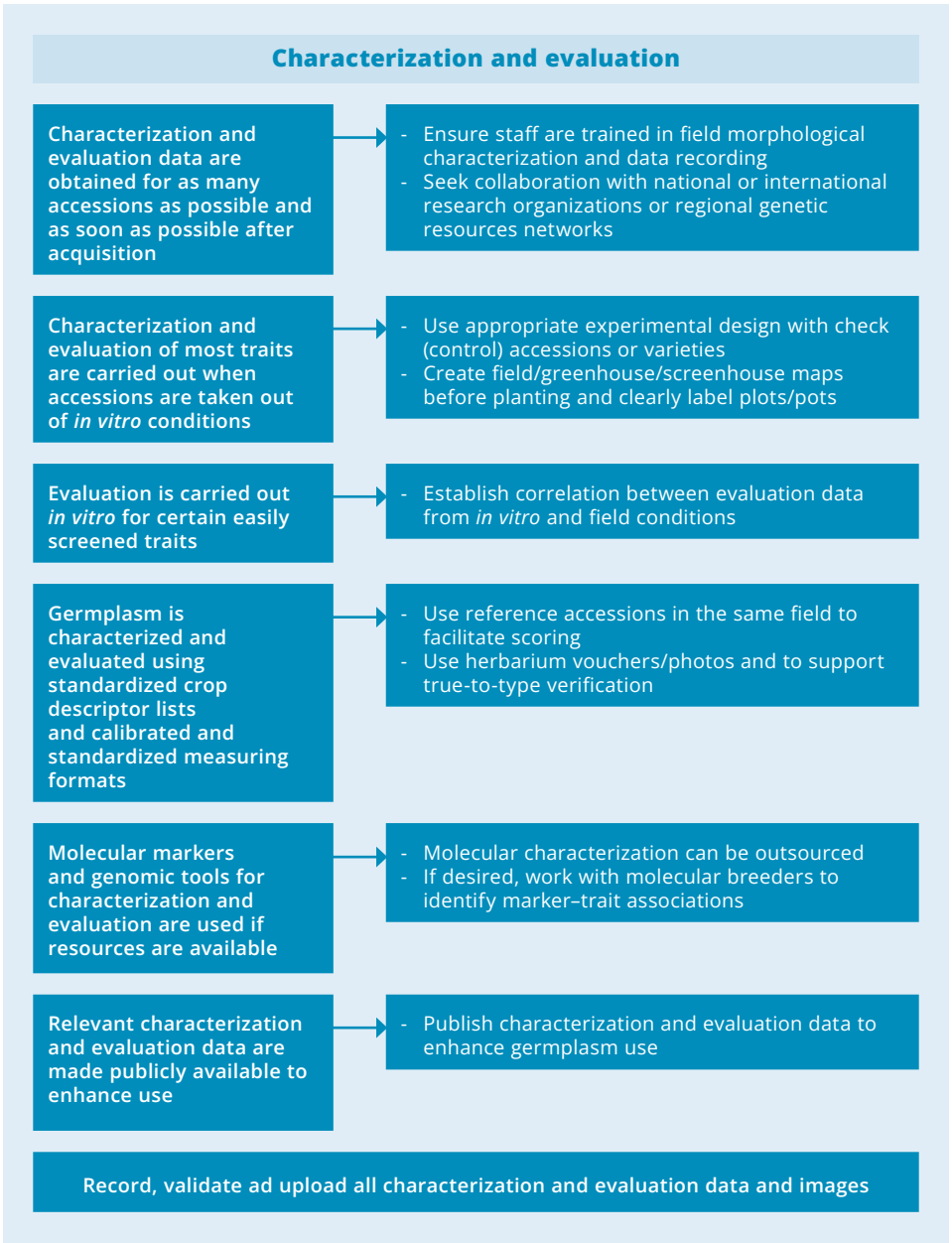
✔ **Molecular marker technologies and genomic tools for characterization are utilized if resources are available, complementing phenotypic characterization.**
Molecular markers help ensure the identity of plants and help identify mislabelled plants and duplications. They are also highly useful in detecting genetic diversity and parentages within and among accessions. Molecular markers are stable and detectable in all tissues. Molecular marker technologies include DNA-based markers and direct sequencing; determining the best method to use will depend on need and resources.[28] Molecular characterization may be outsourced to specialized laboratories.

✔ **All characterization and evaluation data, including associated metadata, are recorded, validated and uploaded to the genebank information management system.**
Data to consider include descriptor measured and results, date recorded, staff responsible, laboratory techniques (molecular, etc.) and dates carried out. 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.

✔ **Relevant characterization and evaluation data are made publicly available.**
Making selected data publicly available to potential germplasm users at genebank, country, regional and global levels will serve to enhance germplasm use (see documentation section). The publishing of characterization and evaluation data is therefore highly recommended.

[28] A number of resources on the various molecular marker technologies available are available online and in print. Please see Further Information/Reading.

Figure 6. Summary diagram of the workflow and activities for characterization and evaluation



6. Documentation

Barcoding, ICRAF

The genebank is recommended have a documented policy and/or procedure, as applicable, for managing genebank data and information, including data-sharing guidelines.[29]

✔ **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 *in vitro* conservation and use of germplasm, including passport, and *in vitro* culture and slow-growth storage, regeneration, characterization, evaluation and 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) and the use of

[29] See Figure 7 at the end of this section for a summary diagram of the workflow and activities for documentation.

standardized, internationally agreed, crop-specific descriptors for characterization and evaluation[30] facilitate data exchange and comparison of accessions across different countries and institutions. Passport data are ideally available for all accessions in the genebank collection.[31]

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.[32]**
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.

✔ **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

30 See characterization and evaluation section.

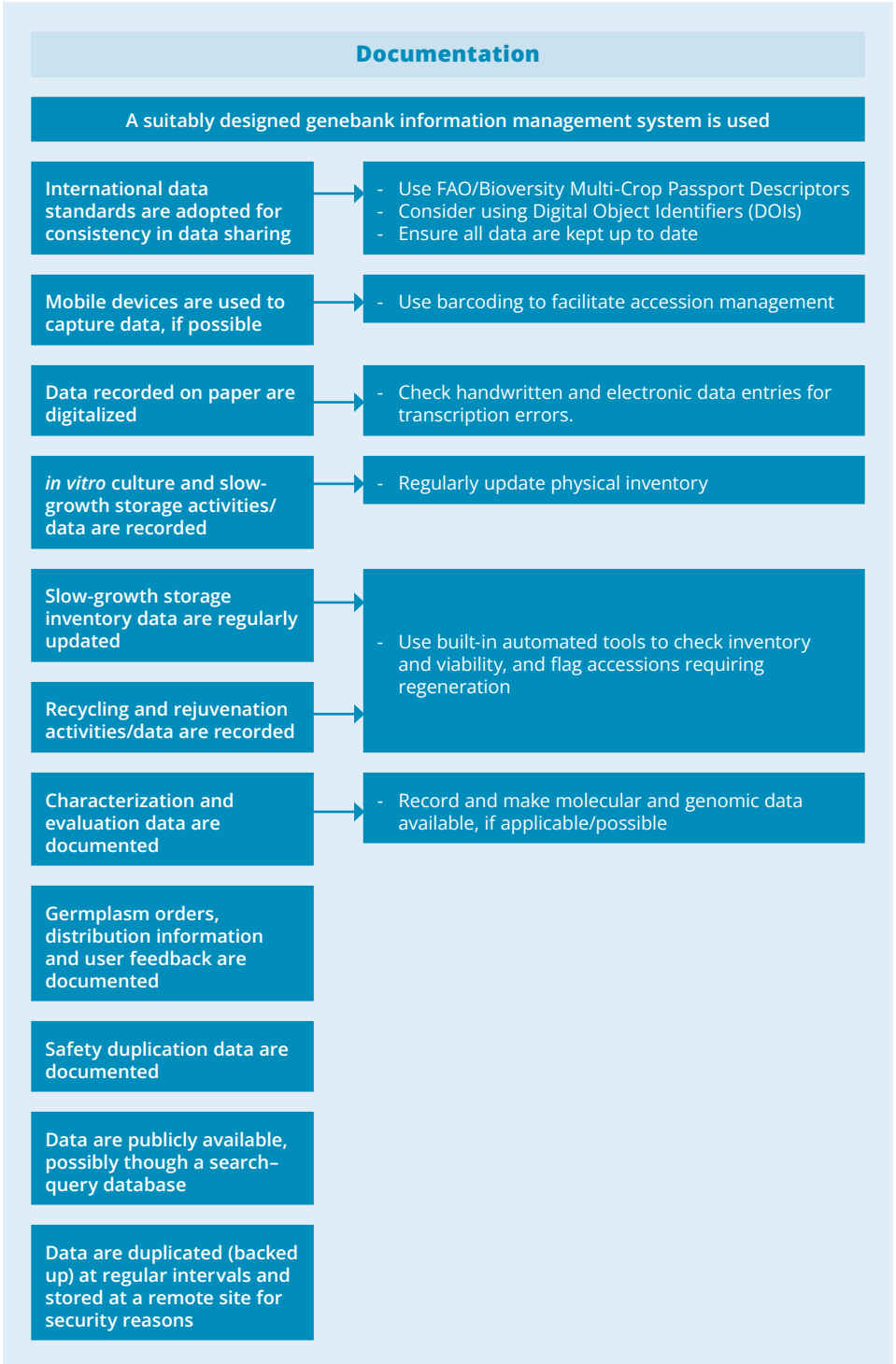
31 Standard 6.6.1.

32 Standard 6.6.3.

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 7. Summary diagram of the workflow and activities for documentation



7. Distribution

***In vitro* distribution of banana germplasm, International Musa Germplasm Transit Centre**

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.[33]

✔ **The genebank complies with national, regional and international regulations and agreements.[34]**

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) is being distributed for the established intended uses (i.e. research, breeding and training for food and agriculture), it is necessary to use a 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

[33] See Figure 8 at the end of this section for a summary diagram of the workflow and activities for distribution of germplasm.

[34] Standard 6.7.1.

or its derivatives, data reporting, etc. An MTA is usually used (e.g. AVRDC, 2012), though an SMTA could also be used.

- ✔ **A policy is in place for the number of plantlets to distribute for any given species.**
  The average size of sample distributed by *in vitro* genebanks is approximately 3–5 plantlets per accession. For accessions with too few plantlets at the time of 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 plantlets is sufficient.

- ✔ **The capacity of the recipient to adequately manage *in vitro* material is assessed, if possible.**
  Ensuring that the distributed germplasm sample will be efficiently used is an important step in managing resources. Often a simple questionnaire will provide the information needed to assess this.

- ✔ **The distributed germplasm is of high quality.**
  It may be necessary to subject material to rejuvenation and/or a decontamination treatment if all replicates are contaminated.

- ✔ **Conditions for the transfer of material are established between the genebank and the recipient and adequate means of re-establishing plants from *in vitro* culture are confirmed.**[35]
  Recipients should have the means to transfer the materials either to pots or to the field. Alternatively, arrangements should be made with other institutes to ensure successful transfer. Genebanks can share information on handling germplasm with recipients to facilitate use.

- ✔ **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 samples and their dispatch is kept to a minimum.**

[35] Standard 6.7.3.

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

✔ **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.**[36]
Consider scanning documents and sending them by email, or sending hard copies by mail, prior to the dispatch of the germplasm. Documentation to consider include:
- data on accessions (including an itemized list with accession identification, number and/or weights of samples, and key passport data); and
- import permit, phytosanitary certificate, or customs declaration, if appropriate.

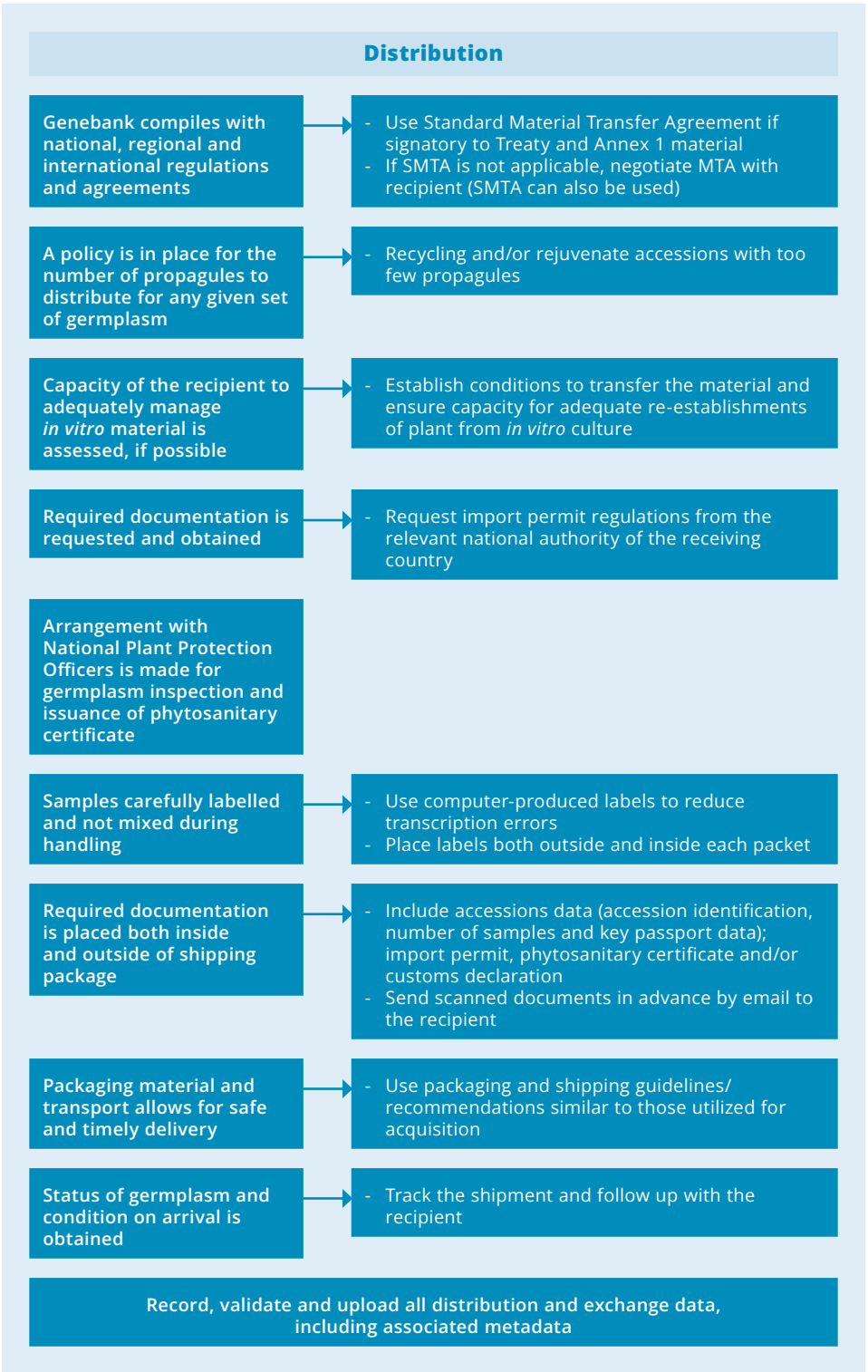
✔ **The choice of packaging material and transport allows for safe and timely delivery.**
Ensure that the material reaches the destination genebank in good condition, bearing in mind the time needed for document processing, duration of shipment, transit time and transit conditions (high temperatures and/or humidity in tropical countries). *In vitro* samples should be placed in sterile, leak-proof plastic bags or sterile transparent watertight sealed plastic vials and packed firmly, but not too tightly, in a box or carton, with the addition of crumpled paper or polystyrene material to protect against shocks.

✔ **The delivery and condition of the germplasm on arrival at its destination is followed up to confirm that germplasm has reached the recipient sufficiently quickly.**
It is suggested that shipments be tracked and that the genebank follow up with the recipient regarding the status and usefulness of the distributed germplasm.

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

[36] Standard 6.7.2.

Figure 8. Summary diagram of the workflow and activities for distribution of germplasm



8. Safety duplication

International Musa Germplasm Transit Centre, Belgium

The genebank is recommended to have a documented policy and/or procedure, as applicable, for the safety duplication 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 shipment schedules.[37,38]

✔ **A safety duplicate sample for every original accession is stored in a distant area, under appropriate conditions and utilizing best practices, and/or backed up by an alternative conservation method/strategy.[39]**
Safety duplicates are deposited at a location well away from the main collection and usually in another country. The safety duplicate location is chosen to minimize possible risks and provide the best possible conditions, taking into account the need for adequate facilities, staff and financial resources. It should be in a sociopolitically and geophysically stable location. The genebank/institute hosting the safety duplicates should have adequate capability to provide appropriate field and/or *in vitro* conditions for the duplicated accessions. Alternatively, samples can be cryopreserved at the duplicating centre.[40] Selection of, and clear agreement with, the chosen holder of the safety duplicate are critical.

37 Duplicated material includes plants to be managed in the field, plantlets maintained *in vitro* or meristematic tissues under cryopreservation.

38 See Figure 9 at the end of this section for a summary diagram of the workflow and activities for safety duplication of germplasm.

39 Standard 5.10.4.

40 See Genebank Standards (Chapter 6).

✔ **A legal agreement setting out the responsibilities of the depositing and the recipient genebanks, and the terms and conditions under which material is maintained and managed, should be in place.**
If the holding genebank does not already have an agreement with another genebank to duplicate the original accessions, consideration should be given to where best they could be duplicated, which will depend on the chosen method of safety duplication.

✔ **The genebank complies with legal, phytosanitary and other regulations and requirements, and each safety duplicate sample is accompanied by relevant associated information.**
Discussions should take place with the host genebank early in the planning process on the required documentation (both for the genebank and the host country), and the applicable customs and quarantine procedures. This will help ensure timely movement of the germplasm.

✔ **The safety duplicate is of high quality and consists of a sufficient quantity of material.**
It is the depositor's responsibility to ensure that the deposited material is of high quality. Best practices include:
- duplicating clean and healthy material;
- subjecting material to rejuvenation and/or a decontamination treatment if required; and
- ensuring that the size of safety-duplicated samples is sufficient to avoid risk of loss.[41]

✔ **Samples are labelled carefully and are not mixed during handling.**
It is important to ensure that samples are correctly labelled, preferably with computer-produced labels to reduce transcription errors in names and numbers.

✔ **The choice of packaging material and transport allows for safe and timely delivery.**
Ensure that the material reaches the destination genebank in good condition, bearing in mind the time needed for document processing, duration of shipment, transit time and transit conditions (high temperatures and/or humidity in tropical countries). The use of packing and shipping guidelines/recommendations similar to those utilized for distribution is recommended (see distribution section).

✔ **Each safety duplicate sample is accompanied by relevant associated information.**[42]
It is recommended that relevant information be sent with the shipment, including an itemized list with accession identification, key passport data, total number of plantlets, type of container, and import permit, phytosanitary certificate or customs

[41] It is recommended to duplicate a minimum of three to five replicates/samples per *in vitro* accession

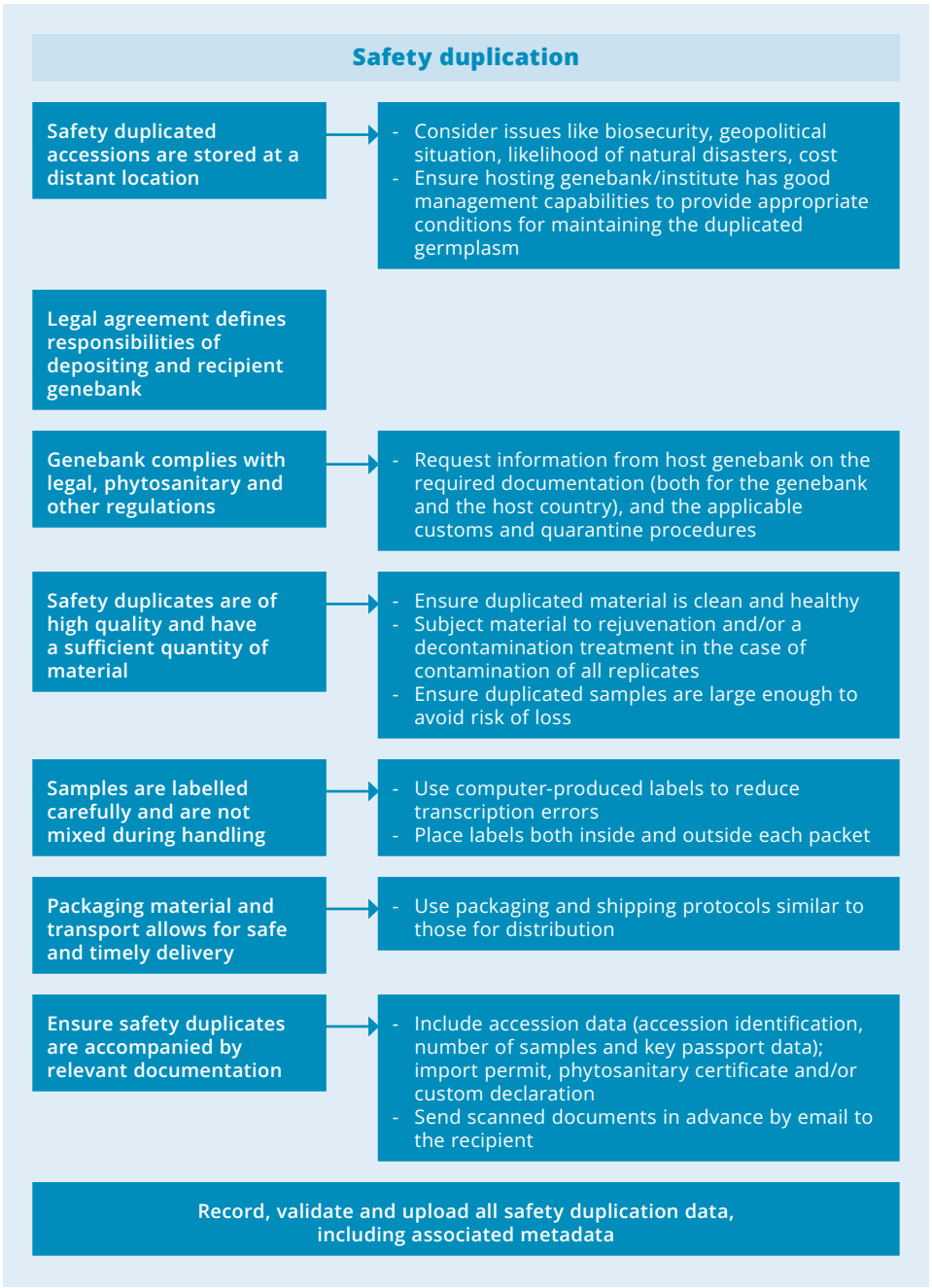
[42] Standard 6.8.5.

declaration, if appropriate.. Consider scanning documents and sending them by email, or sending hard copies by mail, prior to the dispatch of the germplasm.

- ✔ **All safety duplication data, including associated metadata, are recorded, validated and uploaded to the genebank information management system.** Data to consider include: the location of the safety-duplicated accessions, samples sent and number of replicates/plantlets per sample; indexing method, if applicable; shipping log and user feedback; and reference to legal agreement, phytosanitary certificate, etc. Consider the use of electronic devices to avoid transcription errors and for ease of uploading into the genebank information management system. 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.

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

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



9. Personnel and security

Genebank staff, IITA

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.[43]

✔ **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.[44] 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.

[43] See Figure 10 at the end of this section for a summary diagram of the workflow and activities for personnel and security.

[44] Standard 6.8.3.

✔ **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.

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.[45] 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.
- *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.

[45] Standard 6.8.1.

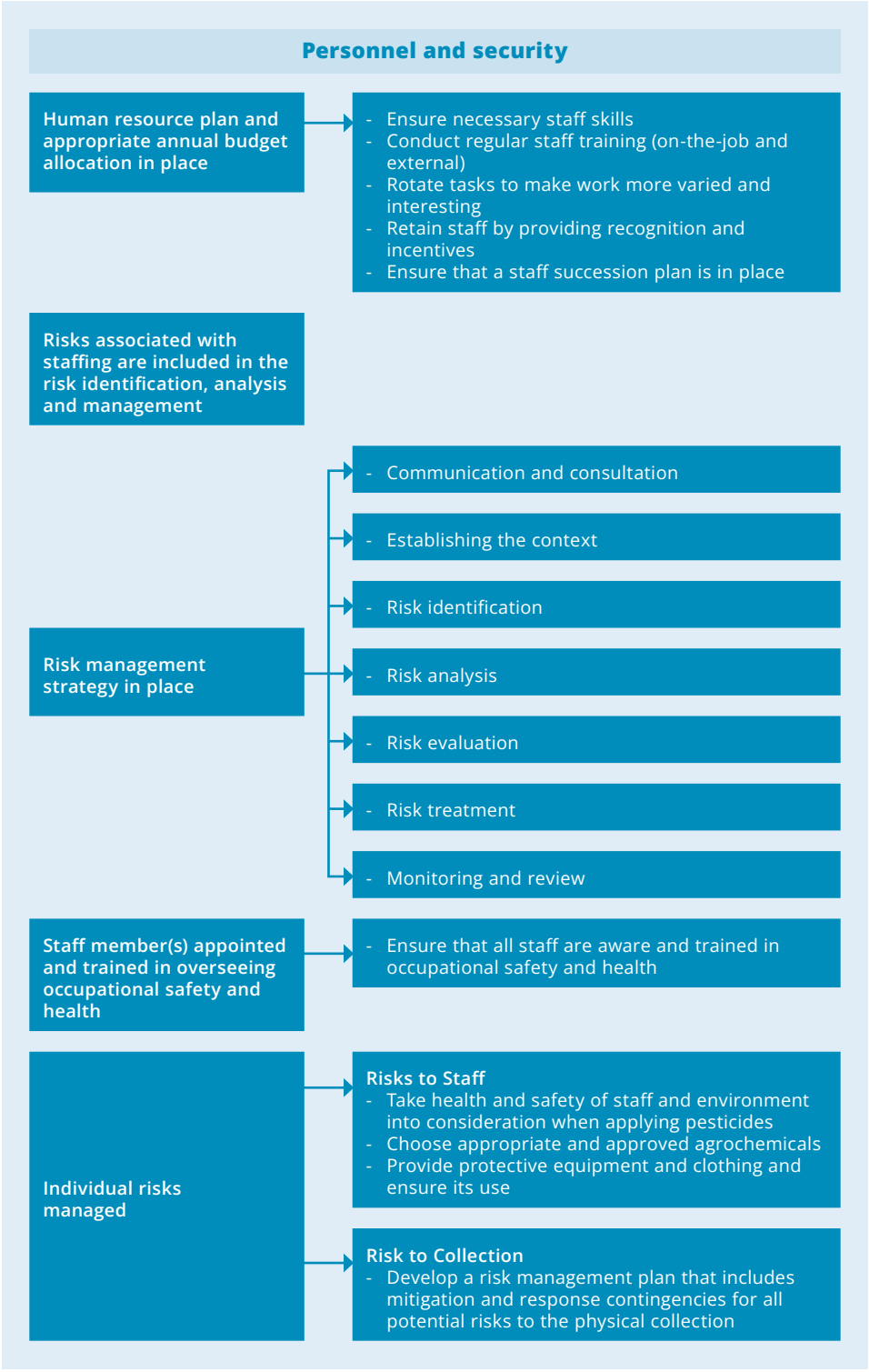
✔ **A staff member with responsibility for occupational safety and health (OSH) in the genebank is appointed and receives training in OSH.**
OSH deals with all aspects of health and safety in the workplace and has a strong focus on primary prevention of hazards.[46] Most countries will have an OSH policy. 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.

[46] Standard 6.8.2.

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



10. Infrastructure and equipment

IPK genebank, Germany

This section considers the suggested infrastructure and equipment for an *in vitro* genebank (Table 2). *In vitro* genebanks are generally equipped with: (a) basic tissue culture equipment, growth rooms and support facilities; (b) specialist storage equipment, such as incubators and acclimatizing chambers; (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.

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 setting up and running *in vitro* facilities, 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 2: General infrastructure and equipment recommended for an *in vitro* 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, moisture retaining bags/containers, labels (ideally barcoded labels), hand lenses, scissors, tarpaulins, secateurs, packaging materials, herbarium presses
Data collection sheets or mobile devices for electronic data recording, GPS or altimeter
Incinerator, surface decontamination solutions, knives, forceps, scalpels, balance for weighing fruit and seeds, camera for recording sample on arrival
***In vitro* culture and slow growth storage**
Autoclave, pH meter, balance, water distillation unit, magnetic stirrer, water bath, automatic pipettes, glassware, chemicals, laminar airflow cabinets, bead sterilizer or burner, fridge/freezer, stereo dissecting microscopes, dissecting instruments, culture medium components, different culture containers, slow-growth media components, temperature-controlled growth rooms, growth room shelving and lights, media for screening for contaminants, antibiotics, fungicides
Recycling and rejuvenation
Field/greenhouse/screenhouse environment and equipment for growing out *in vitro* plants for rejuvenation or to assess changes in morphology
Characterization and evaluation
Access to field, laboratory or greenhouse/screenhouse areas as required
Field/laboratory/greenhouse/screenhouse equipment and machinery, as necessary, according to species and traits being recorded
Pots and plot stakes and labels (ideally barcode labels), labelled cloth bags or other appropriate containers
Molecular analysis (RAPD, ISSR, SSR) equipment, if possible
Data sheets or mobile devices for electronic data recording, barcode reader

Table 2 *(Cont.)*

Genebank operation/management area
Documentation
Suitable designed database/genebank information management system aligned to FAO/Bioversity MCPDs and other data standards, e.g. GRIN-Global
Database with built-in inventory and viability/health monitoring tools, and flagging accessions requiring recycling and rejuvenation
Data backup/storage
Distribution and safety duplication
Sterile plastic bags for distribution of *in vitro* germplasm. Heat-sealable plastic bags and sealing machine, labels (preferably barcoded), packaging 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

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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 follows genetic sampling guidelines
Taxonomic misidentification	■ Include a taxonomist in the collecting team and hire genebank staff trained in taxonomy ■ Take herbarium vouchers and photos for verification by experts
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 longevity	■ Ensure timely transfer to controlled conditions ■ Ensure appropriate post-harvest handling according to propagule maturity, prevailing environmental conditions and phytosanitary conditions

In vitro culture and slow-growth storage

Risk	Risk control/mitigation
Reduced propagule longevity	■ Ensure appropriate media and storage conditions, including disease management
Loss of genetic integrity due to somaclonal variation	■ Avoid the use of excess growth regulators in media ■ Limit the number of subcultures ■ Discard any cultures exhibiting somaclonal variation
Mixing/mislabelling of samples	■ Label carefully to avoid mixing ■ Use computer-generated barcode labels to minimize errors
Stored sample falls below viability or quantity thresholds	■ Ensure that the documentation system includes automated tools to monitor viability and inventory and flag up accessions requiring regeneration

Recycling and rejuvenation

Risk	Risk control/mitigation
Loss of adaptive alleles due to selection pressures	■ Ensure appropriate media and recycling conditions ■ Rejuvenate under controlled environmental conditions
Misidentification of sample/ accession	■ Check container and pot labels; use bar codes

Characterization and evaluation (*in vitro*)

Risk	Risk control/mitigation
Poorly recorded, unreliable data	■ Well-trained staff ■ Mobile devices to record field data ■ Data validation by curator and/or documentation officer
Misidentification of sample	■ Check container labels while collecting data

Characterization and evaluation (greenhouse or field)

Risk	Risk control/mitigation
Poorly recorded, unreliable data	■ Well-trained staff ■ Appropriate statistical design ■ Selection of appropriate locations for planting ■ Appropriate cultural practices ■ Mobile devices to record field data ■ Data validation by curator and/or documentation officer
Misidentification of sample/ accession	■ Use of check accessions/varieties ■ Check plot labels while collecting data ■ Check plot and pot labels prior to sowing and harvesting

Distribution and safety duplication

Risk	Risk control/mitigation
Mixing/mislabelling of samples	■ Careful packaging to avoid mixing ■ Labels placed inside and outside of package ■ Use computer-generated barcode labels to minimize errors
Viability loss due to delayed or damaged shipments	■ Ensure samples are dispatched promptly and use the fastest and safest way of sending.

FAO has developed the *Practical guide for the application of the Genebank Standards for Plant Genetic Resources for Food and Agriculture: Conservation via* in vitro *culture* to be used as a companion volume to the *Genebank Standards for Plant Genetic Resources for Food and Agriculture*. The action steps of the genebank workflow are presented in a sequential manner and provide guidance on the complex steps and decisions required when operating an *in vitro* genebank. The accompanying summary charts for the respective action steps underscore the intended use of this practical guide as a handbook for routine genebank operations for the conservation of plantlets by means of *in vitro* culture. While this practical guide is particularly useful for genebank technicians for their day-to day activities, it may also be used as a basis for the development of standard operating procedures and quality management systems. Genebank managers will also find it useful for conducting training exercises.

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