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COMMISSION ON GENETIC RESOURCES FOR FOOD AND AGRICULTURE

Item 4.1 of the Provisional Agenda

INTERGOVERNMENTAL TECHNICAL WORKING GROUP ON PLANT GENETIC RESOURCES FOR FOOD AND AGRICULTURE

Ninth Session

Rome, 18–20 April 2023

APPLICATION OF THE GENEBANK STANDARDS FOR PLANT GENETIC RESOURCES FOR FOOD AND AGRICULTURE

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I. INTRODUCTION

1. The Commission on Genetic Resources for Food and Agriculture (Commission), at its Fourteenth Regular Session, endorsed the Genebank Standards for Plant Genetic Resources for Food and Agriculture[1] (Genebank Standards), prepared under the Commission's guidance.[2] The Genebank Standards provide guidance on the conservation of plant genetic resources for food and agriculture (PGRFA) *ex situ*, in seed banks, in field genebanks, in *in vitro* cultures and through cryopreservation.

2. At its Seventeenth Regular Session, the Commission requested FAO to prepare practical guides for the use of the Genebank Standards[3]. At its Eighteenth Regular Session, it took note of three practical guides for the application of the Genebank Standards and requested FAO to finalize and disseminate them. The Commission also requested FAO, to develop further additional practical guides on the conservation in genebanks of species producing recalcitrant seeds, and cryopreservation.[4]

3. This document provides an update on the finalization of the three practical guides presented to the last session of the Commission and proposes outlines for the two additional practical guides for the implementation of the Genebank Standards.

II. BACKGROUND

4. The Genebank Standards set the benchmark for current scientific and technical best practices and reflect the key international policy instruments for the *ex situ* conservation and use of PGRFA. They are an important tool for implementing the Second Global Plan of Action for Plant Genetic Resources for Food and Agriculture,[5] which is a supporting component of the International Treaty on Plant Genetic Resources for Food and Agriculture.[6] The Genebank Standards encourage active genebank management, recognizing that the many genebanks around the world differ greatly in the species and size of their collections, as well as the human and financial resources at their disposal.

5. Due to limited capacities and inadequate infrastructure, many countries face challenges in ensuring secure long-term conservation of PGRFA. The purpose of the practical guides is to present the relevant information contained in the Genebank Standards in a format that details the different actions of the genebank workflow in a sequential manner. The practical guides aim to facilitate more widespread application of the Genebank Standards and contribute to the development of an efficient and sustainable system of *ex situ* conservation.

6. As requested by the Commission[7], FAO finalized three practical guides so far, to be used as companion volumes to the Genebank Standards:

- *Practical guide for the application of the Genebank Standards for Plant Genetic Resources for Food and Agriculture*: *Conservation of orthodox seeds in seed genebanks*;[8]
- *Practical guide for the application of the Genebank Standards for Plant Genetic Resources for Food and Agriculture*: *Conservation in field genebanks*;[9] and,

[1] FAO. 2014. *Genebank Standards for Plant Genetic Resources for Food and Agriculture*. Rome. http://www.fao.org/3/a-i3704e.pdf
[2] CGRFA-14/13/Report, paragraph 102.
[3] CGRFA-17/19/Report, paragraph 65.
[4] CGRFA-18/21/Report paragraph 100.
[5] FAO. 2011. Second Global Plan of Action for Plant Genetic Resources for Food and Agriculture. Rome. https://www.fao.org/3/i2624e/i2624e00.pdf
[6] http://www.fao.org/plant-treaty/en/
[7] CGRFA-17/19/Report, paragraph 65.
[8] FAO. 2022. *Practical guide for the application of the Genebank Standards for Plant Genetic Resources for Food and Agriculture*: *Conservation of orthodox seeds in seed genebanks*. Commission on Genetic Resources for Food and Agriculture. Rome. https://doi.org/10.4060/cc0021en
[9] FAO. 2022. *Practical guide for the application of the Genebank Standards for Plant Genetic Resources for Food and Agriculture*: *Conservation in field genebanks*. Commission on Genetic Resources for Food and Agriculture. Rome. https://doi.org/10.4060/cc0023en

- *Practical guide for the application of the Genebank Standards for Plant Genetic Resources for Food and Agriculture*: *Conservation via* in vitro *culture*.[10]

7. For the time being, the practical guides have been published in English only. However, extra-budgetary resources have been secured to translate the practical guides into French. FAO is actively seeking in-kind or extra-budgetary support to make the practical guides available in all United Nations languages.

III. KEY FEATURES OF THE PRACTICAL GUIDES FOR THE CONSERVATION IN GENEBANKS OF SPECIES PRODUCING RECALCITRANT SEEDS AND CONSERVATION THROUGH CRYOPRESERVATION

8. Seeds that do not survive desiccation and freezing, which are the typical processes for storage of germplasm in seed genebanks, are referred to as non-orthodox or recalcitrant seeds. Recalcitrant seeds require specialized handling in the short term. Cryopreservation is a technique for the long-term conservation of biological materials including species producing recalcitrant seeds, at ultra-low temperatures, which halt biochemical and most physical processes.

9. Many globally important plantation crops, fruit trees and agroforestry species produce recalcitrant seeds. The *ex situ* conservation of these species requires specialized knowledge and techniques to ensure their short-term hydrated storage while they are prepared for planting in field genebanks, introduced into *in vitro* culture, or cryopreserved. Their long-term conservation in field genebanks or via *in vitro* culture is even more challenging. In this regard, cryopreservation is becoming increasingly important as a cost-effective and long-term conservation strategy of PGRFA, especially for species that: produce recalcitrant seeds; have exceptional long reproductive cycles; or, can only be propagated vegetatively.

10. The development of practical guides to support the conservation of recalcitrant-seeded species and cryopreservation reflects the increasing importance of these conservation approaches. The proposed draft outlines are presented in Annexes I and II to this document, for consideration by the Working Group.

11. The new practical guides will be structured to align with Chapter 6 of the Genebank Standards and will be in harmony with the three published guides. Each guide will have an introductory section that provides a brief overview of conservation of species producing recalcitrant seeds and cryopreservation, respectively. This section will include a table summarizing the underlying principles of genebank management[11] and their related genebank operations, as well as a flow chart outlining the flow of germplasm for the respective conservation methods.

12. The main sections of both practical guides will provide general guidance for the steps and decisions required for the respective conservation methods, including the key activities outlined in the Genebank Standards. An additional section will provide an overview of basic infrastructure and equipment required. Important sources of information and references will also be included. The practical guide on species producing recalcitrant seeds focuses on their short-term maintenance and steps necessary to prepare the propagules for long-term conservation either in the field, *in vitro* or under cryopreservation. This guide is intended to be used in combination with the other relevant guides.

13. An annex to each Practical Guide will review the risks and associated mitigation for each activity, respectively.

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

[11] The underlying principles of genebank management include: 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.

IV. EXPERT CONSULTATION

14. An expert consultation will be held during the second semester of 2023 to review and discuss the proposed content of the two practical guides. The consultation will include experts in seed physiology, conservation of recalcitrant-seeded species and cryopreservation.

V. GUIDANCE SOUGHT

4. The Working Group may wish to:

- Recommend that the Commission welcome the finalization and publication of the three practical guides published by FAO in 2022;
- Recommend that the Commission request FAO to disseminate the practical guides in all United Nation languages and to call upon donors to provide the necessary funds for this;
- Review the draft outline of the practical guides for conservation in genebanks of species producing recalcitrant seeds and for conservation through cryopreservation, respectively, and recommend that the Commission request FAO to take the comments and inputs of the Working Group into account in the development of these practical guides; and,
- Recommend that the Commission invite FAO to convene a virtual expert consultation on the draft practical guides and further develop them based on the feedback received from the Working Group, the Commission and the expert consultation, for review by the Working Group at its next session.

Annex 1: Draft outline for the practical guide for conservation in genebanks of species producing recalcitrant seeds

1. Introduction

- General introduction to recalcitrance and seed storage biology
- General aspects of recalcitrant-seeded species ecology
- Major PGRFA species producing recalcitrant seeds
- Long term conservation options for recalcitrant-seeded species

2. Acquisition of germplasm

This section will be harmonized with the three published practical guides but modified for those activities related specifically to the acquisition of recalcitrant-seeded species.

2.1 Germplasm acquired through collecting missions

- Emphasis on collecting, handling and transport of large-seeded species or ripe fruits
- Include policies and procedures for the collection of species from the wild
- Cross referencing with the three published practical guides, to avoid duplication

2.2 Germplasm acquired through transfer/donation

3. Testing for non-orthodox behaviour and assessment of water content, vigour and viability

- Use of available predictive and modelling tools:
  - Ecological correlates and predictive models for recalcitrant behaviour
  - Population modelling to characterize level of desiccation tolerance
- Handling materials quickly and under controlled conditions
  - Preparing seeds, for example cleaning to remove soft fruit tissue
  - Reducing the risk of pre-sprouting, fungal contamination, etc.
  - Short-term storage under moist conditions
- Direct methods of determining seed desiccation tolerance
- Determination of water content
- Assessment of seed viability and vigour using optimal environmental conditions

4. Short-term hydrated storage of recalcitrant seeds

- Maintaining germplasm health
  - Pros/cons of surface disinfection
- Determining environmental conditions based on species
  - Oxygen requirements
  - Moisture requirements
  - Temperature requirements
  - Storage container needs
- Pre-sprouting by storing at temperatures that are suboptimal for germinations.
- Regular monitoring of seed quality

5. Preparing recalcitrant seeds/ propagules for conservation in genebanks

5.1 Field genebanks

- Planting recalcitrant seeds from germination test
- Planting recalcitrant seeds immediately upon arrival
- Planting recalcitrant seeds from short-term hydrated storage
- Field genebank planning and implementation

- Relevant sections of the practical guides for conservation in field genebanks will be cross-referenced to avoid repetition.

5.2 *In vitro* culture and slow-growth storage

- Germinate recalcitrant seed propagules as a source of shoot-tip explants
  - Determining best practices for germination
  - By product from germination testing
- Follow best practices for initiation into in vitro culture
  - Relevant sections of the practical guides for conservation via *in vitro* culture will be cross-referenced to avoid repetition.

5.3 Cryopreservation

- Isolating embryos or axes from recalcitrant seeds
- Follow best practices for initiation into cryopreservation
  - Relevant sections of the practical guides for conservation through cryopreservation will be cross-referenced to avoid repetition.

5.4 Pollen banking

- Desiccation tolerance assessment of pollen
- Separating bi- vs tri-nucleate pollen based on difficulty of handling
- Pollen viability assessments
- Desiccation tolerant pollen
- Packaging for pollen banking
- Determining storage temperature

6. Documentation

This section will be harmonized with the three published practical guides and modified as required for activities related specifically to the documentation of the activities required for the conservation of recalcitrant-seeded species. Relevant sections of the practical guides on conservation in field genebanks, via *in vitro* culture and through cryopreservation will be cross-referenced to avoid repetition.

7. Distribution

This section will be harmonized with the three published practical guides and modified as required for activities related specifically to the distribution of recalcitrant seeds.

8. Personnel and security

This section will be harmonized with the three published practical guides

8.1 Personnel
8.2 Security

9. Infrastructure and equipment

This section will focus on the infrastructure and equipment required for the activities required for activities related specifically for the conservation of recalcitrant-seeded species, especially for short term hydrated storage. Relevant sections of the practical guides on conservation in field genebanks, via *in vitro* culture and through cryopreservation will be cross-referenced to avoid repetition.

10. References cited

11. Further information / reading

12. Annex: Risks and associated mitigation

The annex will focus on the risks and associated mitigation related to the activities required for activities related specifically for the conservation of recalcitrant-seeded species. Relevant sections of the practical guides on conservation in field genebanks, via *in vitro* culture and through cryopreservation will be cross-referenced to avoid repetition.

Supporting material: Each section will include a summary diagram of the workflow and activities associated with that section. Tables and Figures will accompany the above sections as necessary.

Annex 2: Draft outline for the practical guide for conservation through cryopreservation

1. Introduction

- General introduction to cryopreservation and its use for long-term conservation
- Species conserved under cryopreservation
    - Need for species-specific methodologies
- Explants/propagules used for cryopreservation
- Current state of cryopreservation of PGRFA

2. Acquisition of germplasm

This section will be harmonized with the three published practical guides and modified as required.

2.1 Germplasm acquired through collecting missions

2.2 Germplasm acquired through transfer/donation

3. Assessment of water content, vigour and viability

Relevant sections of the practical guides on conservation of recalcitrant-seeded species will be cross referenced to avoid duplication.

- Maintaining propagules at their harvested water content
    - Handling materials quickly and under controlled conditions
    - Determining propagule water content individually
- Determining desiccation tolerant of orthodox seed and pollen before cryopreservation
- Assessment of propagule viability and vigour using optimal environmental conditions
- Assessment of regenerative ability of propagule

4. Preparing propagules for introduction into cryopreservation

Number of propagules to be cryopreserved and withdrawn for quality assessment. Based on the known regeneration level with a predetermined protocol using a probabilistic model.

4.1 Pre-dried propagules

a. Orthodox seed

- Achieving optimum moisture content
- Packaging for use at ultra-low temperatures

b. Pollen

- Methods for pollen processing
- Equilibrate to 50 percent RH
- Packaging

4.2 Hydrated propagules

- Reducing the water content of hydrated propagules such as dormant buds, shoot tips, cell cultures and recalcitrant seed explants (embryos or axes)
- Controlled freezing
    - Preconditioning of encapsulated shoot tips
    - Chemical vitrification of culture cells and encapsulation-vitrification
    - Freeze desiccation
    - Deep cooling

5. Viability monitoring during storage

Rewarming all propagules removed from storage at appropriate rates to minimise the risk of ice crystallisation (hydrated propagules) or limit expansion stress (dried propagules). Relevant sections of other practical guides will be crossed-referenced as necessary.

5.1 *Pre-dried propagules*

a) Orthodox seed

- Monitoring intervals determined
- Germination testing based on optimized and documented procedures.

b) Pollen

- Monitoring intervals determined
- Optimal conditions used for pollen viability testing
    - Agar plates or *in vitro* culture for the species are used to assess the production of long pollen tubes
    - stored pollen quality is tested using vital stain (e.g. FDA)
    - fertilization of flowers (*in vivo*) to assess subsequent seed set

5.2 *Hydrated propagules*

- Removal of cryoprotectants
- Rewarming propagules in a warm water bath and transferred to *in vitro* culture
- Environmental conditions for regrowth are adjusted to enable culture material to recover
- Dormant buds are grafted onto rootstock.

6. Post-cryopreservation quality assessment

- Quality assessment is based on the regeneration of whole plants (normal phenotype and true-to-type) from all propagules cryopreserved
- Quality assessment of pollen is based on successful *in vivo* fertilization and germination of seed produced
- Determination of genetic integrity
- Assessment of viral load
- Assessment of microbiome

7. Regeneration

- Accessions are regenerated optimally when viability or quantity falls below the respective thresholds
- Direct replanting to obtain seed
- Germination of seeds to obtain shoot tips from seedlings (example tree species)
- Retrieval from field genebanks or *in vitro* culture

8. Characterization and evaluation

These activities will be carried out in the field/greenhouse or, for some traits, during *in vitro* culture/slow-growth conditions. Relevant sections of the practical guides for conservation in seed genebanks, field genebanks and via *in vitro* culture will be cross-reference to avoid repetition.

9. Documentation

This section will be harmonized with the three published practical guides and modified as required for activities related specifically to the documentation of the activities required for cryopreservation. Relevant sections of the practical guides for conservation in seed genebanks, field genebanks and via *in vitro* culture will be cross-referenced to avoid repetition.

10. Distribution

This section will be harmonized with the three published practical guides and modified as required for activities related specifically to the distribution of cryopreserved samples.

11. Safety duplication

This section will be harmonized with the three published practical guides and modified as required for activities related specifically to the safety duplication of cryopreserved accessions. Relevant sections of the practical guides for conservation in seed genebanks, field genebanks and via *in vitro* culture will be cross-referenced to avoid repetition.

12. Personnel and security

This section will be harmonized with the three published practical guides

12.1 Personnel
12.2 Security

13. Infrastructure and equipment

This section will focus on the infrastructure and equipment required for the activities required for activities related specifically for cryopreservation. Relevant sections of the practical guides for conservation in seed genebanks, field genebanks and via *in vitro* culture will be cross-referenced to avoid repetition.

14. References cited

15. Further information / reading

16. Annex: Risks and associated mitigation

The annex will focus on the risks and associated mitigation related to the activities required for activities related specifically for cryopreservation. Relevant sections of the practical guides on conservation in seed genebanks, field genebanks, via *in vitro* culture will be cross-referenced to avoid repetition.

Supporting material: Each section will include a summary diagram of the workflow and activities associated with that section. Tables and Figures will accompany the above sections as necessary.