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COMMISSION ON GENETIC RESOURCES FOR FOOD AND AGRICULTURE

Item 4.2 of the Provisional Agenda

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

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APPLICATION OF THE GENEBANK STANDARDS FOR PLANT GENETIC RESOURCES FOR FOOD AND AGRICULTURE: PRACTICAL GUIDES FOR (1) CONSERVATION IN GENEBANKS OF SPECIES PRODUCING NON-ORTHODOX SEEDS AND (2) CONSERVATION THROUGH CRYOPRESERVATION

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I. INTRODUCTION

1. The Commission on Genetic Resources for Food and Agriculture (Commission), at its Nineteenth Regular Session, welcomed the finalization and publication of the three practical guides developed by FAO to facilitate the implementation of the Genebank Standards for Plant Genetic Resources for Food and Agriculture (Genebank Standards).[1] Noting ongoing work on two further practical guides, addressing the conservation through cryopreservation and the conservation in genebanks of species producing recalcitrant seeds, the Commission recommended to convene a virtual expert consultation on these guides and, based on the feedback received, develop them further for review by the Working Group at its next session.[2]

2. This document briefly introduces the two newly developed draft Practical Guides, for consideration by the Working Group. The two Draft Practical Guides are presented in the documents *Draft Practical Guides for the Application of the Genebank Standards for Plant Genetic Resources for Food and Agriculture: Conservation in genebanks of species producing non-orthodox seeds*[3] and *Draft Practical Guides for the Application of the Genebank Standards for Plant Genetic Resources for Food and Agriculture: Conservation through cryopreservation.*[4]

II. BACKGROUND

3. The Commission, at its Fourteenth Regular Session, endorsed the Genebank Standards,[5] prepared under the Commission's guidance.[6] 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. The Genebank Standards constitute an important tool for implementing both the International Treaty on Plant Genetic Resources for Food and Agriculture[7] (the International Treaty) and the Second Global Plan of Action for Plant Genetic Resources for Food and Agriculture[8] (Second GPA).

4. At its Seventeenth Regular Session, the Commission requested FAO to prepare practical guides for the use of the Genebank Standards.[9] In response to this request, FAO, in consultation with the Global Crop Diversity Trust and technical experts, initiated the development of practical guides for the application of the Genebank Standards. At its Eighteenth Regular Session, it took note of three practical guides addressing: the conservation of orthodox seeds in seed genebanks; conservation in field genebanks; and conservation via *in vitro* culture. The Commission also requested FAO, to develop further additional practical guides, especially for the conservation in genebanks of species producing recalcitrant seeds, and for cryopreservation.[10]

5. The purpose of the practical guides is to present the information contained in the Genebank Standards in a more user-friendly format that details the different actions of the genebank workflow in a sequential manner and thereby facilitate more widespread application of the Genebank Standards. The practical guides are underpinned by the underlying principles of all genebank management,[11] as

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[1] 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; 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; 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.
[2] CGRFA-19/23/Report, paragraph 52.
[3] CGRFA/WG-PGR-12/24/4.2/Inf.1.
[4] CGRFA/WG-PGR-12/24/4.2/Inf.2.
[5] FAO. 2014. *Genebank Standards for Plant Genetic Resources for Food and Agriculture*. Rev. ed. Rome. http://www.fao.org/3/a-i3704e.pdf
[6] CGRFA-14/13/Report, paragraph 102.
[7] http://www.fao.org/plant-treaty/en/
[8] http://www.fao.org/agriculture/crops/thematic-sitemap/theme/seeds-pgr/gpa/en/
[9] CGRFA-17/19/Report, paragraph 65.
[10] CGRFA-18/21/Report, paragraph 100.
[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.

outlined in chapter 2 of the Genebank Standards. Genebanks may use the activities outlined in these guides as a basis to develop Standard Operating Procedures and Quality Management Systems for conserving germplasm collections, defining in detail how each activity is carried out.

6. The two newly developed draft practical guides have been developed in collaboration with over 20 technical experts. Virtual expert consultations were held in October 2024, bringing together participants from all FAO regions and representing national research institutes, botanical gardens, academia, CGIAR, Crop Trust and the Secretariat of the International Treaty.

III. CONSERVATION IN GENEBANKS OF SPECIES PRODUCING NON-ORTHODOX SEEDS

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

8. However, a small proportion of species produce seeds that cannot be described as orthodox. This may be because

(i) they do not tolerate drying to low moisture content (3–7 percent f.wt.) and low-temperature (<0 °C) storage, in which case the seeds are described as recalcitrant; or

(ii) they only tolerate partial drying (to 10–12 percent moisture content) and/or show an anomalous response to temperature, in which case the seeds are described as intermediate.

9. It is important to note that there are also some species which produce seeds that are orthodox in the sense that they tolerate low moisture content and storage at <0 °C, but they may be described as 'intermediate' since their longevity is very short under conventional orthodox genebank conditions (at most, a few years). These are not discussed further here, but species which produce short-lived orthodox seeds would, like species which produce intermediate or recalcitrant seeds, be candidates for alternative methods of *ex situ* conservation (in field genebanks, *in vitro* and/or in cryogenic storage).

10. Not all species have documented seed storage behaviour. It has been predicted that overall, 92 percent of angiosperm species will produce orthodox seeds. It is also recognized that recalcitrant seed storage behaviour has only been documented within certain plant families). But if receiving or collecting seeds of species for which seed storage behaviour has not been documented and non-orthodox behaviour is suspected, the genebank is encouraged to do research to characterize the seed storage behaviour.[12] Whether the storage behaviour is known or not, it may be helpful or necessary to maintain some seeds under 'hydrated' storage. These are conditions under which seeds neither take up (imbibe) or lose (dry) moisture.

11. The draft practical guide for the conservation of species producing non-orthodox seeds is structured to align with the relevant sections of Chapter 6 of the Genebank Standards[13] and makes linkages to the previously published practical guides to avoid duplication.[14] It provides detailed information on the actions and best practices for acquisition of germplasm, short-term storage

[12] See section 5.
[13] Chapter 6: Genebank standards for *in vitro* culture and cryopreservation. The Draft Practical Guide for the Application of the Genebank Standards: Conservation in genebanks of species producing recalcitrant seeds does not provide details on *in vitro* culture or cryopreservation.
[14] 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; 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; 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

under humid conditions for seeds known to be recalcitrant, short- to medium-term storage of intermediate seeds, testing of seeds with unknown but suspected non-orthodox seed storage behaviour, preparing material for long-term conservation, documentation, distribution of intermediate seeds, safety duplication, and personnel and security. Each section is supported by a summary diagram of the actions in sequential order. In addition, the guide considers the suggested infrastructure and equipment for designing or modifying the facilities of a seed genebank to handle non-orthodox seeds. The final section refers to literature providing further guidance and/or technical background on conserving species producing recalcitrant seeds. An annex identifies the potential risks associated with the different genebank operations and proposes measures to address them.

IV. CONSERVATION THROUGH CRYOPRESERVATION

12. Plant genetic resources that are cryopreserved, stored at liquid nitrogen temperatures in a living state, have the potential to remain viable for decades without requiring regeneration. Cryopreservation technologies have been developed and implemented in numerous genebanks to preserve materials, particularly for security back-ups, that would be expensive to replicate in other forms, such as duplicates in field, greenhouse/screenhouse, or *in vitro* conditions.

13. Storing materials in cryotanks cooled by liquid nitrogen is not the most cost-effective storage method for standard orthodox seeds that survive for decades or even centuries in freezer storage at -18ºC.[15] However, cryopreservation can be particularly beneficial for use with propagule types that cannot be successfully stored under dried/frozen conditions. Although it may be labour-intensive and expensive to introduce materials into cryostorage, the annual cost of their preservation in liquid nitrogen is often much less than the cost of maintaining duplicate field, greenhouse/screenhouse, and *in vitro* plant collections, or regenerating seeds frequently due to their short storage life.

14. Genebanks that employ cryopreservation technologies to secure collections often have a single location dedicated for cryopreservation activities, rather than duplicating the unique facility requirements at multiple dispersed locations. This arrangement requires careful coordination among multiple genebank sites.

15. Some genebanks rely on cryopreservation as a preferred method to store back-ups of collections that are maintained in another form; however, if materials are lost at the active sites, the cryopreserved backup may be the only remaining material. Other genebanks may strategize to only use liquid nitrogen to preserve accessions for some collections. This is particularly applicable for some recalcitrant and intermediate seed collections for which there may not be corresponding field collections in the genebank network.

16. The draft practical guide for conservation through cryopreservation is similarly structured to align, as possible, with Chapter 6 of the Genebank Standards[16] and refers to sections of the previously published practical guides to avoid duplication.[17] It provides key considerations for cryopreservation, detailed information on the actions and best practices on acquisition of germplasm, cryopreservation of diverse propagule types, long-term storage in liquid nitrogen, viability/regrowth assessment, monitoring of cryopreserved propagules, regeneration/replacement of cryopreserved inventories, post-cryopreservation quality assessment, documentation, distribution of materials back to active collections or safety backups to original genebanks, safety duplication of cryopreserved samples, and personnel and security. Summary schemes outlining each of the sequential steps required when operating a cryopreservation facility are provided for each of these topics. In addition, the draft practical guide suggests infrastructure and equipment for designing or modifying cryopreservation facilities. The final section comprises a list of references to provide guidance and/or technical background on cryopreservation operations and management. An annex identifies the potential risks associated with the various genebank

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

operations and proposes preventive measures.

V. GUIDANCE SOUGHT

17. The Working Group may wish to review and, as appropriate, revise the draft practical guides for the conservation in genebanks of species producing non-orthodox seeds and for conservation through cryopreservation and recommend their publication and dissemination.