Draft Updated Genebank Standards: Minimum Standards for Conservation of Orthodox Seeds

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1. Introduction

Genebanks around the world hold collections of a broad range of plant genetic resources, with the overall aim of long-term conservation and accessibility of the plant germplasm to plant breeders, researchers and other users. A sustainable conservation of these plant genetic resources depends on effective and efficient management of genebanks through the application of standards and procedures that ensure the survival and availability of plant genetic resources at present and in the future. For any conservation effort to be sustainable and successful it should also be cost effective and well managed.

The D*raft Updated Genebank Standards* arise from the revision of the FAO/IPGRI *Genebank Standards*, published in 1994. The revision was undertaken at the request from the Commission on Genetic Resources for Food and Agriculture (CGRFA) in light of the changes in the global policy landscape and advances in field of science and technology. The main policy developments that impact the conservation of plant genetic resources in genebanks lie within the context of availability and distribution of germplasm arising from the adoption of international instruments such as Convention on Biological Diversity (CBD), the International Treaty on Plant Genetic Resources (ITPGRFA) and the International Plant Protection Convention (IPPC). On the scientific front, advances in seed storage technology, biotechnology , and information and communication technology (ICT), have added new dimensions to plant germplasm conservation.

The *Draft Updated Genebank Standards* are minimum standards and are concerned solely with the conservation of seeds of orthodox species, including wild species, i.e. those species whose seed can survive considerable desiccation, and in which longevity can be improved by reducing seed storage moisture content and/or temperature. The standards are underpinned by a set of broad underlying principles that provide the overarching framework for an effective and efficient management of genebanks. These key principles at the core of a genebank operation are the preservation of germplasm identity, maintenance of viability and genetic integrity, promoting access including the associated information to facilitate use of the stored plant material in accordance with relevant national and international regulatory instruments. The standards provide the specificity to ensure that a genebank can adhere to these underlying principles.

It is noted that these standards are voluntary and nonbinding and have not been developed through a formal standard-setting procedure. They should be viewed as targets for developing an efficient, effective, rational and transparent global system of *ex situ* conservation that provide optimal maintenance of seed viability and genetic integrity in genebanks, thereby ensuring access to and use of high quality seeds of conserved plant genetic resources. They take into account the diversity in storage requirements and the variable economic circumstances of genebanks holding national, regional and international collections.

These standards do not cover *ex situ* conservation of transgenic seeds as they have legal and technical implications that are as yet unresolved. Nor should these standards be used for non- orthodox seeds or clonally propagated crops. Appropriate standards for such collections will be developed in due course.

The *Draft Updated Genebank Standards* can be aimed at by all genebanks for conserving orthodox seed collections, butthey should not be used uncritically because there are continuous technological advances in conservation methods, much of it species-specific, as well as in the context of the purpose and period of germplasm conservation and use. It is therefore recommended that the *Draft Updated Genebank Standards* be used in conjunction with other reference sources, particularly references to species-specific information.

This document is divided into three parts, namely the Underlying Principles, Standards and the Appendices. The standards are detailed in nine sections and a selective list of references is provided for all the standards.

2. Underlying principles

Genebanks globally share many of the same basic goals, but their missions, resources, and the systems they operate within often differ. As a result, curators have to optimize actively their own overall genebank system and this objective requires management solutions which may differ substantially across institutions, but that would still lead to the same goals. Underlying principles explain why and for what purpose plant genetic resources are being conserved. These principles provide the basis for establishing the norms and standards essential for the smooth operation of a genebank. The major underlying principles for conservation are described in the section below.

Identity of accessions

Care should be taken to ensure that the identity of seed sample accessions conserved in genebanks is maintained throughout the various processes, beginning with acquisition through to storage and distribution. Proper identification of seed samples conserved in genebanks is closely related to careful documentation of data and information about the material. This will begin with recording passport data in the case of material acquired through collecting activities, or donor information where material has been acquired through donations; information should also be recorded for older collections in the genebanks for which passport data is not available or not recorded earlier. When genebanks collect crop wild relatives and other economically important wild species, correct identification of such material is also dependent on voucher specimens being taken at the time of collecting and maintained in a herbarium. Modern techniques such as bar-coding accessions can greatly facilitate the management of the germplasm without incurring errors and thus ensuring the identity of the accessions in question.

Maintenance of viability

Maintaining viability and genetic integrity of seed samples and making them available for use is the ultimate aim behind genebank management. It is, therefore, critically important that all processes adhere to the minimum standards necessary to ensure that acceptable levels of viability are maintained. A high initial viability can ensure the attainment of a maximum period of conservation under long-term conditions, thus decreasing the frequency of regeneration to avoid loss of alleles. To meet these aims, particular attention needs to be paid to standards on germplasm acquisition, processing and storage. In general, seed samples accepted into the genebank at the point of acquisition should have high viability and as far as possible meet the minimum requirements. Collecting the seeds as close as possible to the time of natural dispersal can ensure the highest physiological seed quality. A monitoring system should be in place to check viability status of stored samples at appropriate intervals depending on expected seed longevity. Costly regeneration can be avoided or at least delayed if correct attention is paid to post-harvest handling, drying and storage.

Maintenance of genetic integrity

The need to maintain genetic integrity is closely related to maintenance of viability and diversity of the original collected sample. All genebank processes, starting from collecting and acquisition through to storage, regeneration and distribution, are important for the maintenance of genetic integrity. Adequately representative seed samples of good quality and sufficient quantity should be obtained during acquisition. Ensuring that viability is maintained above minimum standards contributes to the maintenance of genetic integrity. To minimize genetic erosion it is important to follow recommended protocols for regenerating seed accessions, with as few regeneration cycles as possible of particular crop species, sufficiently large effective population sizes, as well as pollination control. A special mention is made here on the importance of safety duplication to respond to risks that can occur in genebank facilities.

Maintenance of seed health

Seeds should be free from quarantine seed-borne diseases and pests. Samples collected or acquired and samples harvested from regeneration/multiplication plots should be tested for quarantine seed-borne diseases and pests. Some infected/infested samples can be easily cleaned. For some other quarantine diseases and pests, the samples should be destroyed or made disease-free through complex and usually expensive methods. Samples processed for storage should be free from quarantine diseases and pests to ensure the reliable distribution of accessions to requestors.

Availability and use of germplasm

The conserved material must be available for current and future use. It is, therefore, important that all processes in genebank operations and management contribute to this goal. There will be a need to maintain minimum standards of seed viability, conserve sufficient quantities of seed, develop germination protocols and generate and document data and related information on the accessions. If information on the conserved germplasm is made easily available and accessible it will enhance its use. Additionally, this will help the genebank curators to plan the activities in the genebank, e.g. monitoring the seed viability, regeneration of less quantities of seeds, characterization of germplasm and testing the genetic stability of the regenerated accessions conserved in the genebank.

Availability of information

In order to ensure communication of information and accountability, essential, detailed, accurate, and up-to-date information at all stages should also be recorded, including historical as well as current information, especially in relation to the management of individual accessions, subsequent to their acquisition. Access, availability and sharing of this information should be treated with high priority, as it leads to better and more rational conservation.

Proactive management of genebanks

Sustainable and effective conservation of genetic resources depends on active management of genebanks. It includes adherence to the legal and regulatory frameworks at national and international levels, in particular as they relate to access, availability and distribution of materials and plant and seed heath. A Standard Material Transfer Agreement (SMTA) should be used in all cases under the Multilateral System of the ITPGRFA. The IPPC regulations provide the framework for quarantine and health regulations to prevent the introduction and spread of plant pests. Proactive management is critical for ensuring that germplasm is efficiently conserved and made available for further use by plant breeders, farmers, researchers and other users. It emphasizes the importance of securing and sharing material as well as the related information, and sets in place a functional strategy for management of human and financial resources for a rational system. It includes risk management strategy and encourages a participatory role of genebanks in the efforts to conserve biodiversity.

3. Standards – structure and definitions

The standards are defined as **minimum standards**. A minimum standard defines that lowest level of performance of a routine genebank operation below which there is an unacceptably high risk of losing genetic integrity (e.g. a probability of five percent or more of losing an allele in an accession).

Each section is divided into

- **A.** Standards
- **B.** Context
- **C.** Technical aspects
- **D.** Contingencies
- **E.** Selected references

The minimum Standards are detailed in nine sections: acquisition, seed drying and storage, viability monitoring, regeneration, characterization, documentation, distribution, safety duplication and security/personnel.

The **Context** provides the basic necessary information in which the minimum standards apply. It provides a brief description of the routine genebank operation for which the minimum standards are defined and the underlying principles for them.

The **Technical Aspects** explain technical and scientific principles important to understand and underpin the minimum standards.

The **Contingencies** provide recommendations in the case that standards cannot be applied to a given species, for example exceptions, alternative routes, and risk management options.

Selected sources of information and references are provided in all sections.

3.1. Standards for acquisition

A. Minimum standards

3.1.1. All seed samples added to the genebank collection have been acquired with relevant legal documentation.
3.1.2. Seed collecting is made as close as possible to the time of natural seed dispersal to ensure maximum seed quality.
3.1.3. To maximize seed quality, the period between seed collecting and transfer to a controlled drying environment is as short as possible.
3.1.4. All seed samples are accompanied by a minimum of associated data: Latin binomial, harvest date, date received by genebank, collecting site, country of origin, donor institution, unique donor identity code (if applicable).
3.1.5. The minimum sample size of a seed sample is its effective population size (Ne).

B. Context

Acquisition is the process of collecting or requesting seeds for inclusion in the genebank, together with related information. The material acquired should be legal, of high seed quality and properly documented.

Acquisition is made in accordance with relevant international and national regulations such as phytosanitary/quarantine laws, ITPGRFA or CBD access regulations, national laws for genetic resources access, and others. Adherence to Standard 3.1.1 will allow the export of seeds from the origin/donor country and the import into the country of the genebank, and determine the management and distribution regime (for example SMTA or bilateral Material Transfer Agreements (MTAs)).

There is a need to ensure maximum seed quality and avoid conservation of immature seeds and seeds that have been exposed for too long to the elements. The way that seeds are handled after collection and before they are transferred to controlled conditions is critical for seed quality. High temperatures and humidity during the post-collecting period and during transport to the genebank could cause rapid loss in viability and reduce longevity during storage. The same applies to post-harvest handling within the genebank. The seed quality and longevity is affected by the conditions experienced prior to storage within the genebank.

During the acquisition phase, it is important to ensure that passport data for each accession is as complete as possible and fully documented. Passport data are crucial in identifying and classifying the accession and will function as an entry point in selecting and using the accession.

C. Technical aspects

For material collected outside the genebank country, there must be a Material Acquisition Agreement (MAA) or Access and Benefit Sharing Agreement (ABSA) drafted and signed by the authorized person in the country of collecting, and according to the national laws for genetic resources access for the country where the collecting will take place (ENSCONET, 2009). Phytosanitary regulations and any other import requirements must be sought from the relevant national authority of the receiving country. For material donated both from within and external to the genebank country, the provisions for the donation, if any, should be made explicit i.e. as SMTA or other type of MTA.

Seeds that are freshly harvested from the field may have a high water content and need to be ventilated to prevent fermentation. They should be placed into suitable containers that allow for good air circulation, and that ensure the contents do not become moist through inadequate air exchange and are neither mixed nor damaged during collecting and transport. Monitoring the temperature and relative humidity (RH) to ensure that seeds are not exposed to conditions above 30 °C or 85 percent RH after collecting and transport, as well as during post-harvest processing will help to maintain seed quality. If fully mature seeds need to be processed and dried in the field, reference should be made to technical recommendations for the particular or similar species to reduce the risk of deterioration.

During collecting appropriate collecting forms should be used. These forms should include information such as the initial taxonomic classification of the sample, the global positioning system (GPS) coordinates of the collecting site, a description of the habitat of the collected plants, the number of plants sampled etc. If possible, the multi-crop passport descriptors should be used (FAO/IPGRI, 2001). In case of donation of the seeds (from a seed company, research programme or genebank), the taxonomic classification, donor, identification number of the donor, and names in addition to the available passport data should be provided. Adequate information about how the germplasm received was maintained should be sought from the donor. Seeds should be assigned a unique identification number (either temporary or permanent, according to the practice used in the genebank) that accompanies the seeds at all times, and that will link the seeds to the passport data and any other collected information, and guarantee the authenticity of the seed sample. Whenever possible a herbarium voucher specimen collected from the same population as the seed samples should be taken, and a record should be made of the method and reason for acquisition.

The sample should represent as much diversity as possible and standard operating procedures for collecting in different environments should be followed. In the ideal case, the amount of seed received from a donor or collected in the field should allow capturing at least 95 percent of the alleles in the sampled population, and - if possible - be so large as to make an initial multiplication unnecessary, but it is recognized that it is not always possible especially in the case of donations of wild species' seeds. Collections from wild populations are sometimes divided by maternal lines to provide additional utility of the sample. Germplasm banks should strive to package and monitor an accession subdivided in this fashion.

D. Contingencies

Collecting should not take place without meeting the legal requirements especially if the germplasm is taken out of the country of collecting afterwards.

Seeds collected in the field are rarely in such condition (physiological and phytosanitary status) that long-term conservation is automatically guaranteed. Multiplication in controlled conditions for the specific purpose of long-term conservation is recommended.

Multiple harvests should be carried out during the genebank's field season to avoid immature seeds and to bias the phenology of the collected sample.

When collections contain a significant proportion (>10 percent) of immature seeds or fruits, measures should be taken to encourage post-harvest ripening. This can usually be achieved by holding material in well ventilated, ambient conditions protected from rainfall. Visual improvements in maturity should be monitored and the material should be transferred to controlled drying conditions as soon as the collected seeds are deemed more mature.

Allowances will have to be made for rare species where seeds might not be available in optimal conditions or quantity.

E. Selected references

[1] **CGIAR** Crop Genebank Knowledge Base (http://cropgenebank.sgrp.cgiar.org)

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[3] **Engels, J.M.M. & Visser L.** eds. *A guide to effective management of germplasm collections*. IPGRI Handbooks for Genebanks, No. 6. IPGRI, Rome, Italy, 2003.

[4] **ENSCONET** *Seed Collecting Manual for Wild Species*, ENSCONET. 2009. ISBN: 978-84-692-3926-1 (www.ensconet.eu).

[5] **FAO/IPGRI.** 2001. *FAO/IPGRI Multi-Crop Passport Descriptors*. FAO, Rome, 4 pp.

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[13] RBG, Kew, Millennium Seed Bank Technical information sheet 04: post-harvest handling of seed collections: http://www.kew.org/msbp/scitech/publications/04-Post%20harvest%20handling.pdf

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[15] **Upadhyaya H. D. & Gowda C.L.L.** 2009. *Managing and enhancing the use of germplasm – Strategies and methodologies*. Technical Manual no. 10. International Crops Research Institute for the Semi-Arid Tropics. 236 pp. Patancheru 502 324, Andhra Pradesh, India.

3.2. Standards for drying and storage

A. Minimum standards

3.2.1. All seed samples are dried to equilibrium in a controlled environment of 5-20 °C and 15-25 percent of relative humidity.
3.2.2. After drying, all seed samples to be sealed in a suitable air-tight container prior to storage at the chosen temperature and relative humidity of 15 percent ± 3 percent.
3.2.3. Most-original-samples and safety duplicate samples should be stored under long-term conditions at a temperature of -18 ± 3 °C. Working samples may be stored under medium-term conditions (under refrigeration, i.e. 5-10 °C) or short-term (under ambient conditions, i.e. 15-25 °C).

B. Context

Maintaining seed viability is a critical genebank function that ensures germplasm is available to users and is genetically representative of the population from which it was acquired (i.e. the most-original-sample). A critical objective of seed drying and storage standards is to reduce the frequency of regeneration of the most-original-sample by maximizing seed longevity, thereby reducing the cost of genebanking and the risks of genetic erosion. For this purpose, long-term storage is required for all most-original samples and for safety duplication of the collection (see Standards for safety duplication). In addition storage standards are also required for circumstances where the objective is to store seeds over the medium- or short-term to keep them alive long enough for distribution to users and evaluation of germplasm. In such cases the standard need not be as stringent as in the case of long-term conservation.

Prior to storage, seed samples need to be dried to appropriate moisture content. A variety of methods can be used for seed drying, the most common being the use of a desiccant or using a dehumidified drying chamber. The methods chosen will depend on the available equipment, number and size of the samples to be dried, local climatic conditions and cost considerations. However, there is a limit to which drying can increase longevity. At a critical moisture level, maximum longevity for the storage temperature is attained and drying below this level does not increase seed longevity further. To realize the full benefit of refrigerated or freezer storage, it is recommended that genebanks dry seeds to the critical moisture level (see Technical aspects). Various RH-temperature combinations can be used during drying, with faster drying possible at higher temperatures but the potential for physiological aging reduced by lower drying temperatures.

Long-term storage conditions as recommended above are expected to provide high seed quality for about 100 years for seed of most agronomic species; medium-term storage conditions are adequate for 30 years and will generally require refrigerated storage. Short-term storage is expected to provide high quality seed for at least eight years and may be accomplished at ambient temperatures (under as cool

and stable temperatures as possible but not more than 25 °C) for some longer-lived species if relative humidity is controlled. It should be pointed out that the longevity of mature, high quality seeds may vary among species and even among seed lots of the same species (Probert *et al*. 2009; Nagel and Börner 2009; Crawford *et al*. 2007; Walters *et al*. 2005). The variation among species and among seed lots of the same species, particularly if seeds are harvested with variable maturity, requires the genebank curator's vigilance to monitor viability (see Standards for viability monitoring).

As seed equilibrium moisture content varies depending on oil content, the best metric for the drying standard is equilibrium relative humidity (eRH) which is constant depending on the relative humidity and temperature of the drying environment. However, it should be noted that in sealed containers during storage, seed eRH will fall or increase if the storage temperature is lower or higher than the drying temperature.

C. Technical aspects

Seed longevity is determined by interactions of biological factors intrinsic to the seed and the quality and consistency of the storage environment, namely the storage temperature and the control of seed moisture content (equilibrium relative humidity). It is well known that seed longevity increases as the seed moisture content and storage temperature decreases, within limits (Ellis and Roberts, 1980; Harrington, 1972). Studies have demonstrated that drying seed beyond a certain critical seed moisture content provides little or no additional benefit to longevity (Ellis *et al*. 1995; Ellis and Hong, 2006) and may even accelerate seed-aging rates (Vertucci and Roos 1990; Walters, 1998). The storage standards as presented are intended to ensure that seeds are stored at this optimum moisture content. However, it has been shown that lowering the storage temperature increases the optimum seed moisture content level (Walters and Engels, 1998; Ellis and Hong, 2006), which suggests there might be danger of over-drying seeds. Conversely, there are reports of successful long-term storage of seeds under 'ultra-dry' conditions (Pérez-García *et al*. 2009). However, there is still uncertainty and requires further research (Ellis and Hong, 2006; Vertucci and Roos 1990; Walters, 1998).

Drying conditions that achieve the critical moisture level at the storage temperature should be determined using water sorption isotherms which show the relationship between the amount of water in the seeds, usually expressed as a percentage of the total seed weight, and their RH. There could be different combinations of relative humidity and drying temperature for given species. Isotherm relationships, predicted based on seed oil content, are available online at the Kew Seed Information Database (SID) website (see references). Genebank operators should clearly understand the relationship between relative humidity and storage temperature to be able to decide about the best combination for their seed drying environment.

After drying, seed moisture should be maintained using moisture-proof containers. Either glass containers that are sufficiently thick to avoid breakage or laminate packaging with a metal foil layer

thicker than 15 μm will maintain desired moisture levels for up to 40 years, depending on the ambient relative humidity at the genebank's location and the quality of the seal. Seed moisture content or eRH should be measured periodically to confirm that storage moisture is adequately maintained.

The storage temperature defines the maximum longevity possible for a seed sample and a stable storage environment is critical to maintaining seed viability. However, there is limited data from long-term storage at a range of low temperatures. Storage at -18 °C has been recommended in the past for long-term storage as it is the lowest temperature that can be achieved with a single stage standard deep freezer compressor. For long-term stored seeds, all attempts should be made to maintain storage temperatures within ±2 ^{o}C of the set temperature and to limit the total duration of fluctuations outside this range to less than one week per year. Genebanks should maintain records of storage temperature deviations and periods when seed accessions are removed from the storage environment. For short-term storage, the seeds should be dried at the same temperature as they are stored, e.g. if ambient condition is 20 °C, seeds should then be dried at that same temperature.

D. Contingencies

Seeds in long-term storage should be removed rarely and only when samples in medium-term storage are exhausted. Desired storage conditions are not achieved when mechanical environmental controls fail or when seeds are repeatedly removed from controlled storage environment. Back-up generators with an adequate fuel supply should be available on-site.

All containers leak and seed moisture will eventually equilibrate to environmental conditions within the storage vault. This occurs faster in containers for which thermal plastics are used as the moisture barrier or if glass or foil laminate containers have faulty seals or imperfections. Seeds may need to be re-dried occasionally and containers or gaskets replaced within 20-40 years.

If clear (for example, glass) containers are used, perforated transparent plastic sachets containing self-indicating silica gel, equilibrated to the drying environment, can be used to monitor container performance during long-term storage. A change in colour of the silica gel inside the sachet (stored alongside the seeds) will indicate moisture ingress if the container seal fails.

Orthodox seeds with short life spans or seeds with low initial quality may deteriorate more rapidly in storage and not meet long-term storage standards unless cryogenic conditions are used.

E. Selected references

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[7]**Kew Seed Information Database**: predict seed viability module (http://data.kew.org/sid/viability/percent1.jsp)

[8]**Kew Seed Information Database**: convert RH to water content (http://data.kew.org/sid/viability/mc1.jsp) and convert water content to RH (http://data.kew.org/sid/viability/rh.jsp)

[9] **Nagel, M. & Börner A.** 2009. The longevity of crop seeds stored under ambient conditions. *Seed Science Research*, 20: 1-12.

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3.3. Standards for seed viability monitoring

A. Minimum standards

3.3.1. The initial seed viability test is conducted after cleaning and drying the accession or at the latest within 12 months after receipt of the sample at the genebank.

3.3.2. Viability monitoring test intervals to be set at one-third of the time predicted for viability to fall to 85 percent[1]. If this deterioration period cannot be estimated and accessions are being held in long-term storage at -18 °C in hermetically closed containers, the interval should be ten years for species expected to be long lived and five years or less for species expected to be short lived.

3.3.3. The viability threshold for regeneration or other management decision such as re-collection is 85 percent. If the initial germination is less than 90 percent, then regenerate/recollect at first detectable significant decline established by an appropriate statistical test.

B. Context

Good seed storage conditions maintain germplasm viability, but even under excellent conditions viability declines with period of storage. It is therefore necessary to assess viability periodically. The initial viability test should be conducted as early as possible before the seeds are packaged and enter the genebank, and subsequent tests are conducted at intervals during storage. If for practical reasons of workflow and efficiency the initial viability test cannot be made prior to banking, it should be made as soon as possible and not later than 12 months after banking. This can be the case of multi-species genebanks, where a wide range of germination regimes is required and samples of the same species are tested all together once a year.

The purpose of viability monitoring is to detect loss in viability during long-term storage before viability has fallen below the threshold for regeneration. The important guiding principle is one of active management of the collection. Too frequent monitoring will result in unnecessary waste of seeds and resources. On the other hand, significant viability decline may not be detected if monitoring is delayed or infrequent; advanced aging of the sample may result in genetic changes (random or directed selection), unrepaired mutations fixed in the sample, or ultimate loss of the accession.

A management decision should be triggered when it is predicted that viability will fall to 85 percent before the next scheduled retest.

[1] The time for seed viability to fall can be predicted for a range of crop species using an online application based on the Ellis/Roberts viability equations (see http://data.kew.org/sid/viability/)

Risk of genetic erosion during storage is lower for homogeneous samples and germination decline to less than 85 percent is allowable as long as plant establishment during regeneration remains adequate. For wild species, landraces, etc. the 85 percent standard should be adhered to.

Models to predict seed longevity from ambient to freezer conditions are available for diverse agricultural species. Genebank staff should use available predictive tools documented for particular species and storage conditions to anticipate duration that seeds will maintain high viability and to guide other genebanking operations such as viability monitoring and regeneration frequencies (see Standards for viability monitoring and regeneration). Longevity predictions based on general species characteristics should be considered as estimates with large confidence intervals. Genebanks are encouraged to develop and report new information that describes and updates species responses to storage conditions.

C. Technical aspects

Viability monitoring intervals should be adjusted according to the data received from germination tests. As soon as a significant decline is detected, monitoring intervals should be reduced in order to 'fine tune' the prediction of time to reach the viability standard.

Accessions with very high initial viability (> 98 percent) may show a statistically significant decline in viability long before the predicted time for viability to fall to 85 percent and when germination is still well above 90 percent. Regeneration or recollection at this point is probably too soon and unnecessary. However, future retest intervals should be brought forward (for example from ten years to five years) in order to track the decline more accurately.

For accessions of lower quality when the initial viability is between 90 and 85 percent, the accession might be dangerously close to the tipping point when viability declines comparatively rapidly. Such accessions should be managed carefully and the first viability monitoring tests should be after 3-5 years of storage intervals at first. Infrequent (for example ten-year) monitoring might fail to detect rapid deterioration and the viability threshold of 85 percent could be missed with negative consequences to the genetic integrity of the collection

Sample sizes for viability monitoring will inevitably be dependent upon the size of the accession but should be maximized to achieve statistical certainty.

It is difficult to establish a strict standard for the number of seeds for germination tests in genebanks. As a general guideline 200 seeds are recommended to be used for initial germination tests (ISTA, 2008) followed by sequential testing, if the initial germination is less than 90 percent (Ellis *et al*. 1985) during storage. But in practice the actual sample size for germination will depend on the size of the accession, which in general is very limited (between 1 000 to 4 000 seeds depending on whether the species is in-breeding or out-breeding) in genebanks. It is important not to waste valuable seeds more than is required for germination tests. For small accession sizes (as is often the case for wild species) sample sizes of 50 seeds or less could be acceptable. However it must be realized then that there may be a

higher chance of germination being below the threshold. The genebank curator should evaluate the risk of this occurrence.

The germination test should always be used in preference to alternatives such as the tetrazolium test. However, in circumstances where it is not possible to remove seed dormancy, alternative tests may be carried out. It is recommended that germination often be measured at two different times so as to have an idea of fast and slow germinating seeds. Records of the number of abnormally germinating seeds should also be kept. Slower germination and increasing abnormals are often early indicators that deterioration is occurring.

Every effort should be made to germinate all viable seeds in a collection using optimum conditions and appropriate dormancy-breaking treatments where needed. Non-germinated seeds remaining at the end of a germination test should be cut-tested to assess whether they are dead or dormant. Seeds with firm, fresh tissue are likely to be dormant and should be counted as viable seeds.

All data and information generated during viability monitoring should be recorded and entered into the documentation system.

D. Contingencies

It is recognized that viability monitoring is an expensive activity and that genebanks may wish to seek cost-cutting procedures. One such procedure may entail measuring seed quality in a subsample of accessions of the same species grown in the same harvest year. This practice may reveal overall trends on the effect of harvest year on seed quality, but will not reveal genotype x harvest year interactions that are known to be important for seed quality. In the event that subsampling is unavoidable, it should be undertaken with sufficient statistical rigour to ensure usefulness of the data in future analyses. For example, performing germination tests on less than ten accessions may not provide sufficient statistical power to compare accessions harvested in different years. Hence, should a subsampling strategy be used, at least 10 percent of same-species accessions harvested in the same year should be evaluated with a minimum of ten accessions evaluated.

An additional strategy would be to focus retesting on the accessions that gave the lowest viability result in the initial tests. Retest data from these accessions should provide an early warning on the performance of the batch as a whole.

However it is recognized that intraspecific variation among accessions has been observed in some cases, thus there are risks associated with the above strategies, which should be considered. Viability monitoring of accessions of wild species is generally more problematic compared with crop species. Seed dormancy is likely to be much more prevalent and small accession sizes often mean that smaller minimum sample sizes have to be adopted for germination tests, as this will inevitably affect the ability to detect the onset of seed deterioration.

The range of inherent longevity is also wider in wild species with some species from Mediterranean and tropical dryland habitats expected to be extremely long lived and conversely some species from cold, temperate regions expected to be short lived. For the latter, retesting intervals of as few as three years should be considered as well as duplication into cryo-storage as a precautionary measure. In the event that storage conditions are not met (as will occur if there is a prolonged power cut when seeds are stored in refrigeration units), viability will be affected negatively depending on the species, length of disruption and conditions during the disruption. In such an event a disaster plan should be activated. For example some representative samples should be tested immediately following resumption of adequate storage conditions.

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[4] **Ellis, R.H., Hong, T.D. & Roberts, E.H.** 1985. Sequential germination test plans and summary of preferred germination test procedures. *Handbook of seed technology for genebanks: Vol I .Principles and methodology*, Chapter 15, pp 179-206. International Board for Plant Genetic Resources. Rome, Italy.

[5] **Engels, J.M.M. &. Visser, L**. eds. 2003 *A guide to effective management of germplasm collections*. IPGRI Handbooks for Genebanks No. 6. IPGRI, Rome, Italy.

[6] **ENSCONET** manual: http://www.ensconet.eu/PDF/Curation_protocol_English

[7] **Harrington, J.F.** 1972. Seed storage longevity. In: T.T. Kozlowski, ed. *Seed biology, Vol III*, pp.145-245, Academic Press, New York, USA.

[8] **International Seed Testing Association (ISTA).** 2008. *International Rules for Seed Testing*. Bassersdorf, Switzerland.

[9] **Royal Botanical Gardens, Kew** Seed Information Database (SID):at http://data.kew.org/sid/

[10] **Smith, R.D., Dickie, J.D., Linington, S.L., Pritchard, H.W. & Probert, R.J.** 2003. Seed *Conservation: turning science into practice*. Royal Botanic Gardens, Kew. Chapters can be downloaded from: http://www.kew.org/msbp/scitech/publications/sctsip.htm (see chapters 17 and 24).

3.4. Standards for regeneration

A. Minimum standards

3.4.1. The most-original-sample is used to regenerate accessions that have lost viability below the agreed minimum threshold. Original and subsequent most-original samples are not depleted below 50 seeds. Remaining seeds of the "original" and "most-original" samples are archived in long-term storage for reference purpose.

3.4.2. The sample size of a to-be-regenerated accession will depend on the reproductive characteristics of the species and contains a minimum number of plants which can ensure that, with a 95 percent probability, alleles of that accession with at least a 0.05 frequency are retained.

3.4.3. The regeneration has to be carried out in such a manner that the genetic integrity of a given accession is maintained; for example, regenerated material should contain less than 1 percent of contamination arising from geneflow of pollen that originated from other accessions of the same species or from other species.

B. Context

Regeneration is a key operation and an integral responsibility of any genebank that maintains orthodox seeds and is a process that leads to an increase of the stored seeds (also called "multiplication") in the genebank and/or to an increase of the viability of the seeds equal to or above an agreed minimum level (the latter is usually called "regeneration"). An accession that is acquired through collecting or received from a donating genebank and that does not have sufficient seeds for long-term storage (i.e. 1 500 seeds for a self-pollinating species and 3 000 for an out-crossing species) or for which the viability has dropped below an established minimum threshold (i.e. below 85 percent germinability of the stored seeds), will have to be regenerated.

As regeneration is an activity that could easily affect the genetic composition of an accession (and thus its genetic integrity) utmost care is required. Consequently, genebank operators will have to strike a delicate balance between avoiding regeneration as much as possible versus the potential loss of viability and thus, the risk of affecting the genetic integrity of an accession. Active management of the collections will greatly help to decide on the best moment to regenerate

Regeneration should be undertaken with the least possible change to the genetic integrity of the accession in question. This means that in addition to sampling considerations (see paragraph below) of the accession in question we need to pay due attention to the environment in which the activity will be undertaken, as such environment might cause severe selection pressure on the accession. It has been suggested that the regeneration environment should be as similar as possible to that at the collecting site, in particular when a population collected in the wild is being regenerated, in order to minimize genetic drift and shift as well as to produce the best possible quality of seeds.

To preserve the genetic integrity of genebank collections during seed regeneration, it is important that sampling of accessions be carried out efficiently, and that the population or sample to be used for the regeneration process be of sufficient size to maintain as much genetic diversity as is practicable. It is therefore important to be able to distinguish the size of a genebank-accession sample needed to obtain one or more rare alleles with a certain probability, and how this sample will affect the genetic integrity of the accession in terms of changes in allele frequency and inbreeding depression (Crossa, 1995).

The methodology to be used for regeneration might well vary from species to species and depends, among others on the breeding system, pollination efficacy, etc., as these factors relate directly to the underlying principles of genetic integrity and identity of an accession. Therefore, it is of significant importance to collate as much as possible of the relevant biological information related to the species in question. In addition, when possible and meaningful, it is recommended that the regeneration event be used also for the characterization of regenerated accessions.

C. Technical aspects

In order to affect the genetic integrity of accessions maintained in long-term storage as little as possible it is recommended to use seeds from the most-original-sample for regeneration, whereas seeds for multiplication are taken from the working collection, i.e. serial regenerations of up to five cycles of the previously regenerated sample without returning to the most original sample (IPGRI, 2003). Where possible it is recommended to use the most original sample at least twice before its viability drops below the threshold.

It should be noted that in cases where the original collection or donation is a small sample, it is necessary to regenerate immediately following receipt of the material in order to obtain an adequate quantity of seeds for long-term storage. It is important to record the number of the regeneration cycle and enter the information into the documentation system. It is recommended that the receiving genebank always keep some seeds from the initial seed sample for future reference purposes. Even if these original seeds lose their viability, they can be useful in confirming morphology or genotype of later generations of the respective accession.

The size of the seed sample to be used in the regeneration activity has to reflect the genetic composition of the accession, i.e. the reproductive biology of the species in question as well as the degree of homogeneity/heterogeneity of the accession. For this purpose the effective population size (Ne) is a key parameter that will have a bearing on the degree of genetic drift that is associated with the regeneration of the accession. This parameter has been established for individual accessions on the basis of the parameters that allow calculation of Ne (Dulloo, M.E. *et al*. 2008).

To avoid geneflow/contamination it is critically important to use established isolation distances between plots of accessions being regenerated and these might well vary among species. This also applies to self-pollinated species, depending on the regeneration environment. For species that depend on specific pollinators, isolation cages and the corresponding pollinators should be used (Dulloo, M.E. *et al*. 2008).

Contamination and genetic drift/shift can be assessed with morphological, enzymatic or other distinctive traits that can be used as markers (e.g. flower colour; seed colour, etc.), or with molecular markers.

Reference collections (herbarium specimen, photographs and/or descriptions of the original accessions) are essential for conducting the true-to-type verification.

D. Contingencies

The management of a genebank and of a germplasm collection is a multifaceted task in which scientific considerations have to be combined with economical, infrastructural, personnel and other aspects and where an optimum balance must be aspired. However, as already indicated, the underlying principles such as genetic integrity and identity have to be given the highest attention while regenerating accessions. Nevertheless, there will always be a risk management dimension to the curatorship role. Solid biological knowledge of the species in question is a key factor in making the best possible decisions under constrained conditions. Aspects such as sample size, distance between individual accessions and other forms of isolating accessions, respecting established thresholds for viability loss, growing conditions and others, all need to be given due attention when planning the regeneration activity.

In view of this complexity it is not meaningful to look for possible contingencies. In case of emergency it would be advisable to seek advice from experts and/or collaboration with other genebanks that could provide assistance.

E. Selected references

[1] **Breese, E.L.** 1989. *Regeneration and multiplication of germplasm resources in seed genebanks: the scientific background*. Available online at: http://www2.bioversityinternational.org/publications/Web_version/209/

[2] **CGIAR** Crop genebank knowledge base http://cropgenebank.sgrp.cgiar.org

[3] **Crossa, J.** 1995. Sample size and effective population size in seed regeneration of monecious species. In: J.M.M. Engels, R. Ramantha Rao, eds. *Regeneration of seed crops and their wild relatives. Proceedings of a consultation meeting,* 4-7 December 1995. ICRISAT, Hyderabad, India. International Plant Genetic Resources Institute, Rome, Italy. pp.140–143.

[4] **Dulloo, M.E., Hanson, J., Jorge, M.A. & Thormann, I.** 2008. Regeneration guidelines: general guiding principles. In:M.E. Dulloo, I. Thormann, M.A. Jorge & J.Hanson, eds. *Crop specific regeneration guidelines* [CD-ROM]. CGIAR System-wide Genetic Resource Programme (SGRP), Rome, Italy. 6 pp.

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[6] **Engels, J.M.M. & Visser, L.** 2003. *A guide to effective management of germplasm collections.* IPGRI Handbooks for Genebanks No. 6. IPGRI, Rome, Italy.

[7] **Lawrence, L.** 2002. A comprehensive collection and regeneration strategy for ex situ conservation. *Genetic resources and crop evolution* 49 (2): 199-209.

[8] **Rao, N.K., Hanson. J., Dulloo, M.E., Ghosh, K., Nowell, D. & Larinde, M.** 2006. *Manual of seed handling in genebanks*. Handbooks for Genebanks No. 8. Bioversity International, Rome, Italy.

[9] **Sackville Hamilton, N.R. & and Chorlton, K.H.** 1997. *Regeneration of accessions in seed collections: a decision guide*. J. Engels, ed. Handbook for Genebanks No. 5. International Plant Genetic Resources Institute, Rome, Italy.

3.5. Standards for Characterization

A. Minimum standards

3.5.1. 100 percent of accessions are characterized within five years of acquisition or the first regeneration cycle.
3.5.2. Characterization is based on standardized and calibrated measuring formats and characterization data follow internationally agreed standards and are made publicly available.

B. Context

Characterization is the description of plant germplasm. It determines the expression of highly heritable characters ranging from morphological or agronomical features to seed proteins or molecular markers.

Characterization can be carried out at any stage of the conservation process, as long as there are sufficient numbers of seeds to sample. As it is essential that the germplasm being conserved is known and described to the maximum extent possible to assure maximum utilization of the seeds to the users, it should be carried out as soon as possible to add value to any collection.

Passport and characterization data help to define the identity of the accession. Collecting information on phenotypic and morphological descriptors to a significant level of detail and making this information on the conserved seeds easily available and accessible will make the accessions more valuable for breeders and other scientists and enhance its use. The use of a minimum set of phenotypic and morphological descriptors and information on the breeding system, such as those published by Bioversity (http://www.bioversityinternational.org/publications/browse by type. html?Search+Search&p type+11#results), is helpful in this regard. The use of internationally agreed standards for characterization data will increase the usefulness of the published data. It is recognized that molecular markers can increasingly be used for characterization (de Vicente, M.C., *et* al. 2004).

C. Technical aspects

Characterization is time consuming and expensive. Characterization can therefore be combined with multiplication or regeneration. Curators should make all possible efforts to record characterization data at the latest during the first regeneration cycle.

Characteristics and traits for crops are defined by crop experts and/or curators in consultation with genebank managers. A wide range of crop descriptor lists has been developed for example by Bioversity International and also minimum sets of key descriptors have been established for several of these. Furthermore there are regional and national descriptor lists available. Data recording needs to be carried out by trained staff using calibrated and standardized measuring formats as indicated in the internationally agreed and published descriptor lists. The data need to be validated by curator and documentation officers before being uploaded into the genebank database and made publicly available.

It is also recognized that reference collections (herbarium specimens, seed herbarium, photographs) play an essential role for true-to-type identification.

D. Contingencies

Reliability of data might vary among data collectors if they are not well trained and experienced. Therefore trained technical staff in the field of plant genetic resources should be available during the entire growth cycle to record and document characterization data. Access to expertise in taxonomy, seed biology and plant pathology (in-house or from collaborating institutes) during the process of characterization is desirable.

Characterization is very labour-intensive and requires sufficient funding to allow for good quality data.

Carrying out full characterization of accessions during regeneration cycles may reduce the number of accessions which can be regenerated per cycle.

The incidence of pests and diseases can limit the collection of quality data. The determination of some traits like oil or protein content requires laboratory assays which are not always available or could be costly.

E. Selected references

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[2] **Bioversity International.** 2007. Developing crop descriptor lists, Guidelines for developers. Bioversity Technical Bulletin No. 13. Bioversity International, Rome, Italy. 71p. Available from: http://www.bioversityinternational.org/index.php?id=19&user_bioversitypublications_pi1[showUid]=3070

[3] **de Vicente, M.C., Metz, T. & Alercia, A.** 2004. *Descriptors for Genetic Marker Technologies*. International Plant Genetic Resources Institute, Rome, Italy. 30p. Available online at: http://www.bioversityinternational.org/index.php?id=19&user_bioversitypublications_pi1[showUid]=2789

[4] **FAO/IPGRI.** 2001. *Multicrop Passport descriptors*. FAO, IPGRI, Rome, Italy. 4p. Available online at: http://www.bioversityinternational.org/index.php?id=19&user_bioversitypublications_pi1[showUid]=2192

3.6. Standards for documentation

A. Minimum standards

3.6.1. Passport data of 100 percent of the accessions are documented using internationally agreed standards and made available in electronic format.
3.6.2. All data and information generated in the genebank relating to all aspects of conservation and use of the material are recorded in a suitably designed database.
3.6.3. A duplicate of all genebank data and information is established outside the genebank for safety reasons.

B. Context

Information about accessions is essential for the genebank to manage and maintain their collection; it is also important to share this information and make it available publicly for potential germplasm users, and should be attached to any distributed material. Passport data are the minimum data that should be available for each accession to guarantee proper management, and international standards such as the multicrop passport descriptors (FAO/IPGRI 2001) should be used to record passport data. The use of internationally agreed standards will very much facilitate data exchange.

Major advances in information technology and bioinformatics have taken place over the last decade or so and much of it is available online. A majority of genebanks also have access to computers and the internet. This new technology makes it possible to record and exchange data and information efficiently. Ultimately conservation and usability of conserved germplasm are promoted through good data and information management. All data and information generated throughout the process of acquisition, registration, storage, monitoring, regeneration, characterization, evaluation, and distribution should be recorded in a suitably-designed database and employed to improve conservation and use of the germplasm. Such data and information ranges from details of the genetic characteristics of individual accessions and populations to distribution networks and clients.

Documentation of characterization and evaluation data is particularly important to enhance the use of the respective collection and help identification of distinct accessions.

C. Technical aspects

Computer-based systems for storing data and information allow for more comprehensive storage of all information associated with genebank management. The adoption of data standards which today exist for most aspects of genebank data management helps to make the information management easier and to improve use and exchange of data. For example, the FAO/IPGRI List of Multicrop Passport Descriptors

should be used for documenting passport data as it is instrumental for data exchange among different genebanks and countries.

Germplasm information management systems exist, such as GRIN-Global, which have specifically been developed for genebanks and their documentation and information management needs.

Evaluation data is often produced by the users to which seeds have been distributed. The genebank should solicit the user to share the evaluation data, which should then be included in the genebank's documentation system. Such information could address resistances to biotic and abiotic stresses, growth and development features of the accession, quality characteristics of yield etc. Adding this type of information allows more focused identification of germplasm to meet prospective client needs.

D. Contingencies

Lack of documentation or loss of it compromises the optimal use of the seeds or can even lead to their loss, if it impedes planning regeneration properly.

There is no indication for the multilateral system (MLS) in the MCPD, so even if the passport data are documented they do not automatically show what is in the MLS of the ITPGRFA.

E. Selected references

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3.7. Standards for distribution

A. Minimum standards

3.7.1. Seeds are distributed in compliance with national laws and relevant international treaties and conventions.
3.7.2. Seed samples are provided with all relevant documents.
3.7.3. At least 95 percent of the seeds and associated data are readily available for distribution and the remaining after multiplication/regeneration.
3.7.4. The time span between receipt of a request for seeds and the dispatch of the seeds is kept to a minimum.
3.7.5. A sample of a minimum of 30-50 viable seeds is supplied for accessions with sufficient seeds. For accessions with too little seed at the time of request, samples are supplied after regeneration/multiplication, based on a renewed request.

B. Context

Conservation should be linked to utilization. Germplasm distribution is the supply of a representative sample of seed accessions from a genebank in response to requests from plant germplasm users. The CBD and ITPGRFA emphasize this continuum between conservation and sustainable utilization, along with facilitated access and equitable sharing of benefits arising from use.

There is a continuous increase in demand for genetic resources to meet the challenges posed by climate change and changes in virulence spectra of major pests and diseases and this demand has led to wider recognition of the importance of using germplasm from genebanks - which ultimately determines the germplasm distribution. The time between receipt of a request for seeds from a user and the following response and dispatch of seeds (along with relevant information) should be kept as short as possible.

The diversity of the legal systems with respect to their procedural rules governing access to courts and to arbitration, and the obligations arising from international and regional conventions applicable to these procedural rules, is recognized.

The Contracting Parties to the ITPGRFA, in the exercise of their sovereign rights over their Plant Genetic Resources for Food and Agriculture, have established a Multilateral System for a defined number of crops (Annex I list) to facilitate access to Plant Genetic Resources for Food and Agriculture and to share, in a fair and equitable way, the benefits arising from the utilization of these resources, on a complementary and mutually reinforcing basis.

Genebanks that hold working collections should promote the availability of genetic resources for uses including research, breeding, education, farming and repatriation.

The exchange of seeds is not accompanied by the risk of spreading some quarantine diseases and insects, and invasive and exotic weed species which could seriously affect national production.

C. Technical aspects

Germplasm should be distributed in a way that ensures the germplasm reaches its destination in good condition. Environmental conditions can be harmful to the quality of seed during transport therefore seeds should be carefully packed and sealed in airtight envelopes for protection during transit.

Samples to be distributed should comply with the requirements of the quality standards as defined in this document and the requirements of seed health as requested by the recipient country. The distribution should also comply with national regulation laws.

Easy and speedy clearance of shipments from customs offices and plant protection departments will most often necessitate the availability of documents required by the recipient country and the requestor.

Phytosanitary certificate, certificate of donation, certificate of no commercial value and import permit are among the documents required by the recipient country. It is therefore important to maintain and update the list of documents requested by different countries.

The list of the material and associated information (passport data as a minimum) should be provided to the recipient together with any legal agreement related to access and use of genetic resources provided.

It is highly recommended to reduce as much as possible the time between the dispatch and the delivery of the shipment. When seeds are not available responses include a detailed description of the reason, an estimated date when the accession will be available, and alternative accessions that may suit the requestor's needs.

The distributing genebank should insist on the flowback of information about the usefulness of the supplied germplasm.

D. Contingencies

Political decisions/situations and bureaucracy might extend the time span between receipt of a seed request and the distribution of the material. Limitations related to regeneration and/or multiplication of the accessions may also affect/delay the distribution process.

E. Selected references

[1] **Convention on Biological Diversity (CBD).** 1992. http://www.cbd.int/convention/convention.shtml

[2] **CGIAR.** Crop Genebank Knowledge Base: http://cropgenebank.sgrp.cgiar.org

[3] **Engels, J.M.M. & Visser, L.** 2003. *A guide to effective management of germplasm collections*. IPGRI Handbooks for Genebanks No. 6. IPGRI, Rome, Italy.

[4] **FAO/IPGRI.** 1994. Genebank Standards.

[5] **International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA)**: http://www.itpgrfa.net/International/

[6] **Rao, N.K., Hanson, J., Dulloo, M.E., Ghosh, K., Nowell, D. & Larinde, M.** 2006. *Manual of seed handling in genebanks*. Handbooks for Genebanks No. 8. Bioversity International, Rome, Italy.

[7] Standard Material Transfer Agreement (SMTA): http://www.itpgrfa.net/International/

3.8. Standards for safety duplication

A. Minimum standards

3.8.1. A safety duplicate sample for every original accession is stored in a geographically distant area, under the same or better conditions than those in the original genebank.
3.8.2. Safety duplicate samples are stored under a black-box arrangement.
3.8.3. Each safety duplicate sample is accompanied by relevant associated information placed within the box used for the shipment

B. Context

Safety duplication is that of a genetically identical subsample of the accession to mitigate the risk of its partial or total loss caused by natural or human-caused catastrophes. The safety duplicates are genetically identical to the base collection and are referred to as the secondary most original sample (Engels and Visser, 2003). Safety duplication includes both the duplication of material and its related information and these are deposited in a base collection at a different location, often outside the country. The location is chosen to minimize possible risks and provides the best possible storage facilities.

Safety duplication is generally made under a 'black-box' approach. This means that the repository genebank has no entitlement to the use and distribution of the germplasm. It is the depositor's responsibility to ensure that the deposited material is of high quality, to monitor seed viability over time and to use their own base collection to regenerate the collections when they begin to lose viability. The germplasm is not touched without permission from the depositor and is only returned on request when the original collection is lost or destroyed. Recall of the deposit is also possible when it is replaced with newly regenerated germplasm.

Safety duplication should be made for all original seeds collected by the genebank or when only held by the genebank. Seeds which are duplicates from other collections can usually be retrieved from those collections and do not require safety duplication unless there is doubt about their security in the other collection.

Any safety duplication arrangement requires a clearly signed legal agreement between the depositor and the recipient of the safety duplicate that sets out the responsibilities of the parties and terms and conditions under which the material is maintained.

C. Technical aspects

When selecting the location for safety duplication, primary consideration is given to the geographic location and environmental conditions of the location. The geographic location determines the type of climate required for minimum energy input in the maintenance of the cooling facilities. Geologic

formations must ensure low radiation (radioactivity) and stability (low probability of earthquakes). The facility must be situated at an elevation that guarantees proper drainage during seasonal rains and eliminates the risk of flooding in the event of rising sea levels due to global warming. Equally important is economic stability and socio-political certainty. A stable economy guarantees the constant flow of capital inputs for the costly maintenance of facilities, genebank operations and personnel. This is unlikely in the presence of social unrest and political uncertainty. Koo *et al*. (2004) suggest that safety duplicate samples should be located away from the risk of political embargo, military action or terrorism that could disrupt international access.

Samples are prepared for safety duplication in the same way as for the base collection. Conditions should be at least as stringent as those for long-term storage of germplasm in a genebank and the quality of seed preparation (i.e., drying) is important.

An initial viability of 90 percent in the case of heterogeneous populations that are subject to genetic changes and 85 percent for others are usually recommended. Special consideration should be given to wild species with low viability (40-60 percent).

Sample size should be sufficient to conduct at least three regenerations. A safety duplicate of an accession in a seed genebank should contain at least 500 viable seeds for outbreeders and heterogeneous accessions with high diversity and a minimum of 300 seeds for genetically uniform accessions. For accessions with seeds of low viability more seeds are necessary. Storage temperatures should be –18 °C to –20 °C.

The packaging material of seeds for safety duplication should be of trilaminate material using a middle metal foil layer of at least 20 µm thickness and formed into a pouch seamed on all four sides with no gusset. This should provide an adequate water barrier for transport and storage at -18 ^{o}C for at least 30 years.

An outer and inner label should be placed on each packet of seeds to ensure that the germplasm is properly identified.

As the storage conditions for the safety duplicate should be the same or better than that of the base collection, seed viability can be monitored on seed lots of the same accession maintained in long-term storage in the genebank and extrapolated to the safety duplicate if basic standards for storage conditions are met and the same containers are used. In some cases, samples for germination testing may be sent in a separate box with the safety duplicate and monitored for germination by agreement with the depository.

Strong cold-resistant boxes (thick carton or polypropylene boxes) are the best options for transporting and storing seeds. Boxes should be sealed properly. Shipment should consider the fastest means of transport available either by air freight, courier or by land to avoid deterioration of seed quality during transit.

Samples should be renewed from the sender when the viability of the samples in similar storage conditions in the base collection of the sender starts to decline. The samples in black-box storage can be either destroyed or returned to the sender and replaced with a new batch.

D. Contingencies

Viability of samples in black-box storage cannot be checked on arrival in the safety deposit. Most damage in transport will only be detectable later if it decreases the longevity of the seeds. This raises a logistic problem. It is proposed that monitoring samples should routinely be included in the shipment and agreements reached with the recipient institution on monitoring viability or returning samples for monitoring to the sender.

When extrapolating the viability of the safety duplicate from viability monitoring results of the sample in the base collection, some caution should however be taken. Seeds may age at different rates if there is a difference in ambient RH at the two sites and/or differences in extent or frequency of temperature fluctuations, though the average storage temperature is the same.

Issues of liability may occur related to sending samples in sealed black-box conditions. One issue is on liability for contents of the sealed box and handling by customs officers and other authorities for entry into a country. In some cases boxes are opened and special seals are applied by the authorities to confirm that the samples are not medicinal or other prohibited plants. Another issue is that on liability of the recipient institution should material be damaged or lose viability earlier than expected as a result of stress during transit, faulty seal of containers, or temperatures that fluctuate from specified standards. Under the conditions described here, the safety duplicate repository should only be “liable” if the temperature becomes uncontrollable; this should be reported immediately to the primary institution so that they can decide on what action to take. The primary institution should bear full responsibility for transport disasters or uncontrolled moisture.

The standards and technical aspects may be difficult to implement for some species due to the inherent biology of the samples, e.g. short-lived seeds, large-seeded species where space and cost may be limiting.

E. Selected references

[1] **CGIAR.** Crop Genebank Knowledge Base. The page on safety duplication, available on line at http://cropgenebank.sgrp.cgiar.org/index.php?option=com_content&view=article&id=58&Itemid=207&lang=english contains detailed background documents, a list of references and a standard safety deposit agreement template.

[2] **Engels, J.M.M. & Visser L.** 2003. *A guide to effective management of germplasm collections*. IPGRI Handbooks for Genebanks No. 6. IPGRI, Rome, Italy. Available in English (1.4 MB) and Spanish (1.5 MB).

3.9. Standards for security and personnel

A. Minimum standards

3.9.1. A genebank employs the minimum staff complement to fulfil all the routine responsibilities of ensuring that the genebank can acquire, conserve and distribute germplasm according to agreed standards.
3.9.2. A genebank should have a risk management strategy in place.

B. Context

Achieving a genebank's goal of acquiring, conserving and distributing germplasm not only requires that adequate procedures and equipment for germplasm handling be in place, but that properly trained staff be employed to carry out the required work and to guarantee the security of the genebank.

Active genebank management requires well-trained staff, and it is crucial to allocate responsibilities to suitably competent employees. A genebank should therefore have a plan or strategy in place for personnel, and a corresponding budget so as to guarantee that a minimum of properly trained personnel is available to fulfil the responsibilities of ensuring that the genebank can acquire, conserve and distribute germplasm. Access to specialists in a range of subject areas is desirable, depending on the mandate and objectives of each individual genebank. However, staff complement and training will depend on specific circumstances. The health and usefulness of the seeds stored in the genebank depend also on issues related to safety and security of the genebank. Arrangements need to be in place for electricity back-up, fire extinction equipment has to be in place and regularly checked, genebank buildings need to be earthquake-proof if situated in a seismic-prone area, to mention some. A genebank should therefore implement and promote systematic risk management that addresses the physical and biological risks in the every-day environment to which the collections and related information are exposed.

C. Technical aspects

Staff should have adequate training acquired through certified training and/or on-the-job training and training needs should be analysed.

Genebank personnel should be aware of and trained in safety procedures to minimize risks to the germplasm.

The genebank facilities should be constructed so as to withstand natural disasters, such as hurricanes, cyclones, earthquakes, or floods that are known to occur in the location where the genebank has been built.

Storage facilities should be protected with standard security facilities such as fences, alarm systems, security doors and any other system that helps to shield the genebank from burglars and other intruders. Security of the seed collections in the genebank will be enhanced by allowing entry strictly to authorized personnel into the actual storage facilities.

Protective clothing should be provided and used in the storage area. Adequate precautions should be taken and safety equipment, including alarms and devices to open doors from inside drying rooms and refrigerated rooms, should be installed.

Refrigeration will almost certainly be reliant on electrical power and it is therefore necessary that the power supply is adequate and reliable. Failure in power supply can result in complete loss of genebank accessions. Consideration should be given to the provision of a back-up generator that automatically cuts in when the main power supply fails. This will require stockpiling adequate amounts of fuel to run the generator during power cuts.

Monitoring devices for temperature should be available in the drying and storage room to track the actual parameters against time.

It should be considered whether it is better to store seed without refrigeration if refrigeration is inherently unreliable. If refrigeration is to be used to conserve germplasm, it must meet minimum standards as unreliable refrigeration can be far more damaging than non-refrigerated storage.

If refrigeration and/or electric power are unreliable, a facility can be built in the soil at a depth of 10-20 m, where temperature can be averaged at 10 °C. This could be attractive in several tropical regions under no risk of flooding. Drying should be well carried out however, and seeds should be kept in properly-sealed vials.

Fire alarm and fire-fighting equipment is required in the genebank. Most fires begin from faulty electrical circuits and therefore periodic checks should be made on the electrical circuitry to ensure compliance with safety standards. Firefighting equipment will include extinguishers and fire blankets. For areas affected by thunderstorms, a lightning rod should be fitted to the genebank.

D. Contingencies

When suitably trained staff is not available, or when there are time or other constraints, it might be a solution to outsource some of the genebank work.

Unauthorized entry to genebank facilities can result in direct loss of material, but can also jeopardize the collections through inadvertent introduction of pests and diseases and interference in management systems.

E. Selected references

[1] **CGIAR.** Crop Genebank Knowledge Base, Section on risk management: http://cropgenebank.sgrp.cgiar.org/index.php?option=com_content&view=article&id=135&Itemid=236&lang=english

[2] **Engels J.M.M. & Visser, L.** 2003. *A guide to effective management of germplasm collec*tions. IPGRI Handbooks for Genebanks No. 6. IPGRI, Rome, Italy. Available in English (1.4 MB) and Spanish (1.5 MB).

Appendices

Appendix I: List of contributors

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Appendix II: **Glossary** (TO BE COMPLETED)

Appendix III: Descriptors for passport and management parameters (TO BE COMPLETED)

Acknowledgements: (TO BE COMPLETED)