Chapter 4

Genebank standards for orthodox seeds

4.1 Standards for acquisition of germplasm

Standards

4.1.1 All seed samples added to the genebank collection have been acquired legally with relevant technical documentation.

4.1.2 Seed collecting should be made as close as possible to the time of maturation and prior to natural seed dispersal, avoiding potential genetic contamination, to ensure maximum seed quality.

4.1.3 To maximize seed quality, the period between seed collecting and transfer to a controlled drying environ ment should be within 3 to 5 days or as short as possible, bearing in mind that seeds should not be exposed to high temperatures and intense light and that some species may have immature seeds that require time after harvest to achieve embryo maturation.

4.1.4 All seed samples should be accompanied by at least a minimum of associated data as detailed in the FAO/Bioversity multi-crop passport descriptors.

4.1.5 The minimum number of plants from which seeds should be collected is between 30-60 plants, depending on the breeding system of the target species.

Context

Acquisition is the process of collecting or requesting seeds for inclusion in the genebank, together with related information. The material should be legally acquired, be 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, and national laws for genetic resources access. Adherence to Standard 4.1.1 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 (e.g. SMTA or Material Transfer Agreements [MTA]).

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. Unfavourable extreme 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. It is recommended that a germination test be conducted immediately after processing and before pre-storage as a way to determine the quality of the seed collected.

During the acquisition phase, it is important to ensure that passport data for each accession is as complete as possible and fully documented, especially georeferenced data that can help to locate collection sites. Passport data are crucial in identifying and classifying the accession and will function as entry points in selecting and using the accession.

Technical aspects

Access to PGRFA, in the multilateral system of the International Treaty, has to be accompanied with a SMTA. The acquirers should comply with the relevant provisions of the ITPGRFA or the CBD and a MTA should be signed by the authorized person in the country of collecting, according to national laws for access to genetic resources of the country where the collecting will take place (ENSCONET, 2009). In addition, when required by the providing country, the access should be subject to the prior informed consent of the country. Phytosanitary regulations and any other import requirements must be sought from the relevant national authority of the receiving country.

Seeds that are freshly harvested from the field may have 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, technical recommendations for the particular or similar species should be applied to reduce the risk of deterioration.

Appropriate collecting forms should be used to capture collection data. 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 and other relevant data that are important for proper conservation. If possible, the FAO/Bioversity multi-crop passport descriptors should be used (Alercia *et al.,* 2012). Very useful additional information, such as cultural practices, previous generations of seed history and origin, uses etc, can be obtained by farmer interviews when seed is collected from farmer fields/stores. During collecting, the collector should also be sensitive to the depletion of the natural population targeted for collecting. The European Native Seed Conservation Network (ENSCONET) collecting manual recommends that the collecting must not overdraw 20 percent of the total seeds available in a population (ENSCONET, 2009). It may also be useful to repeat sampling from a particular site to maximize capture of genetic variability that may be present at various points in time.

The collection sample should be sufficient to include at least one copy of 95 percent of the alleles that occur within the target population with a frequency greater than 0.05 (Marshall and Brown, 1975). A random sample of 59 unrelated gametes is sufficient to achieve this objective and in a species mating complete at random this equates to 30 individuals whereas in a completely selfing species, this target requires 60 individuals (Brown and Hardner, 2000). Thus, the sample size to capture 95 percent of the alleles can vary between 30 and 60 plants depending on the breeding system of the target species. In practice, adequate quantities of seeds should be collected for distribution in order to avoid frequent regeneration. However, we should recognize that this target may not always be met depending on the availability of seeds for collection.

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, including pedigree or lineage information, as well as chain of custody

information where available. 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.

Contingencies

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

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 in terms of above standards (e.g. sample size) will have to be made for wild and rare species where seeds might not be available in optimal conditions or quantity.

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4.2 Standards for drying and storage

Standards

4.2.1 All seed samples should be dried to equilibrium in a controlled environment of 5–20 °C and 10-25 percent of relative humidity, depending upon species.

4.2.2 After drying, all seed samples need to be sealed in a suitable airtight container for long-term storage; in some instances where collections that need frequent access to seeds or likely to be depleted well before the predicted time for loss in viability, it is then possible to store seeds in non–airtight containers.

4.2.3 Most-original-samples and safety duplicate samples should be stored under long-term conditions (base collections) at a temperature of –18 ± 3 °C and relative humidity of 15 ± 3 percent.

4.2.4 For medium-term conditions (active collection), samples should be stored under refrigeration at 5–10 °C and relative humidity of 15 ± 3 percent.

Context

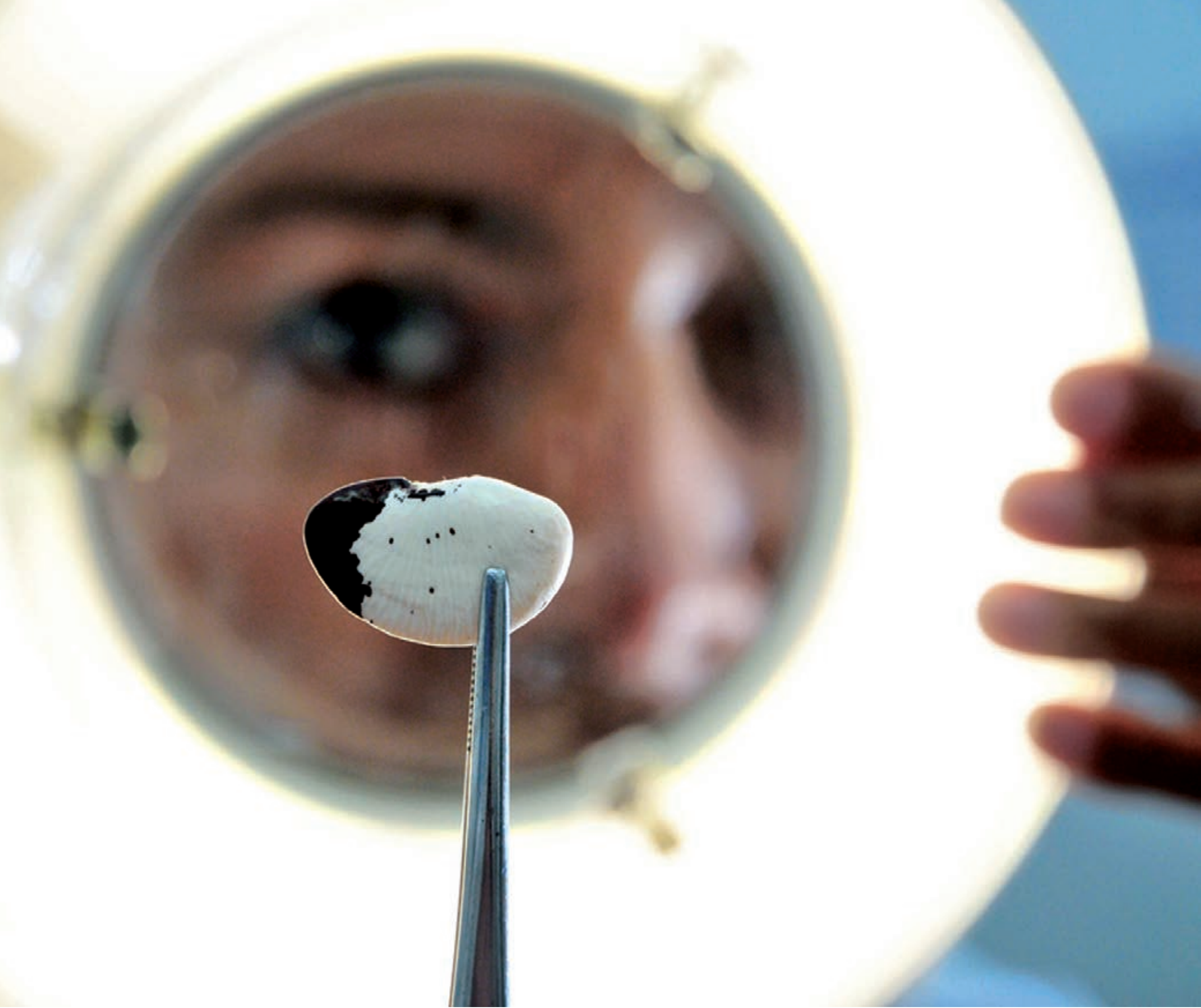
Maintaining seed viability is a critical genebank function that ensures germplasm is available to users and is genetically representative of the population from which it was acquired (i.e. the most-original-sample). A critical objective of seed drying and storage standards is to reduce the frequency 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. 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 long periods, the actual timing is species-specific; 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 RH is controlled according to Standard 4.2.2. 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, 2010; 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 measurement for the drying standard is equilibrium relative humidity (eRH) which is constant depending on the RH 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.



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 (eRH) as well as being species dependent. 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 certain critical seed moisture content provides little or no additional benefit to longevity (Ellis *et al.*, 1985; 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.

Drying conditions that achieve the critical moisture level at the storage temperature should be determined using water sorption isotherms that 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 RH and drying temperature for given species. Isotherm relationships, predicted based on seed oil content, are available online at the Kew SID website (see references). Genebank operators should clearly understand the relationship between RH and storage temperature to be able to decide about the best combination for their seed drying environment.

As soon as the seeds have reached the desired moisture content, they should be packaged and stored. After drying, seed moisture should be maintained using moisture-proof containers. Different types of containers can be used including glass, tin, plastic containers, and aluminium foils, each with their advantages and disadvantages (Gómez-Campo, 2006). In any case, either glass containers that are sufficiently thick to avoid breakage or laminate packaging with a metal foil layer of adequate thickness will maintain desired moisture levels for up to 40 years, depending on the ambient RH at the genebank's location and the quality of the seal. For example, in Germany the genebank uses laminated aluminium foils that are 11µm thick, while the accessions held in Svalbard are held in 20µm laminated aluminium foils. 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 are 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 ±3 °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.

Contingencies

Seeds in long-term storage should be removed rarely and only when samples in medium-term storage are exhausted or seeds need monitoring. Desired storage conditions are not achieved when mechanical environmental controls fail or when seeds are repeatedly removed from controlled storage environment. Backup 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 is not absolute, 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 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.

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4.3 Standards for seed viability monitoring

Standards

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

4.3.2 The initial germination value should exceed 85 percent for most seeds of cultivated crop species. For some specific accessions and wild and forest species that do not normally reach high levels of germination, a lower percentage could be accepted.

4.3.3 Viability monitoring test intervals should be set at one-third of the time predicted for viability to fall to 85 percent[1] of initial viability or lower depending on the species or specific accessions, but no longer than 40 years. 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.

4.3.4 The viability threshold for regeneration or other management decision such as recollection should be 85 percent or lower depending on the species or specific accessions of initial viability.

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/).

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 storage, 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 storage, it should be made as soon as possible and not later than 12 months after receiving. 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.

When it is predicted that viability will fall to 85 percent before the next scheduled retest, the time of the retest should be anticipated or the accession directly scheduled for regeneration.

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 heterogeneous samples such as wild species and landraces, the 85 percent standard should be adhered to. For some landraces, specific accessions, wild species and forest species, a viability of 85 percent in newly replenished seed is rarely achievable. In these situations, the curator can set the viability standard trigger for selected species to a lower threshold, such as 70 percent or lower.

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 genebank 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.

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, 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 (e.g. from ten years to five years) in order to track the decline more accurately.

For accessions of lower quality, the accession might be dangerously close to the tipping point if 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 (e.g. 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. In this respect, the use of statistical models can help to predict the tipping point and predict a time frame for appropriate regeneration.

Viability testing should give the manager an approximation of the viability of the sample. The goal should be to detect differences of +5 percent or so, rather than differences of +0.1 percent. Sample sizes for viability monitoring will inevitably be dependent upon the size of the accession but should be maximized to achieve statistical certainty. However, the sample size should be minimized to avoid wasting seed. Seed in a genebank is a valuable resource and should not be wasted.

It is difficult to establish a strict standard for the number of seeds for germination tests in genebanks. However, standard protocols as outlined by the International Seed Testing Association (ISTA) are often used. As a general guideline, 200 seeds are recommended to be used for initial germination tests (ISTA, 2008). If the initial germination is less than 90 percent, the sequential testing procedure proposed by Ellis *et al.* (1985) can help to save seed in subsequent germination tests during storage. However, in the event that there are not sufficient seeds, 100 or even smaller seed samples are also adequate and should be conducted with replications. The germination test is a guide of viability and even small seed samples can give the manager useful information. But in practice the actual sample size for germination will depend on the size of the accession, which is very limited in general (ideally

the recommended minimum size for self-pollinated is 1 500 and for cross-pollinated species 3 000 seeds) in genebanks. It is important to minimize the use of valuable seeds 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 assess 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 be measured at two different times 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.

Contingencies

It is recognized that viability monitoring is an expensive activity and that genebanks would 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 take genotype by harvest year interactions into consideration that are known to be important for seed quality.

Where different harvest conditions occur over a wide range of maturities across accessions, then a sampling strategy can be from separate harvested sub-groups. 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.

The initial germination test at harvest for known hard-seeded species and accessions frequently found in some forage legume species and Crop Wild Relatives can be as low as 45 percent, and increases after 10–15 years to 95 percent or more and remains so for long periods. If the initial germination is less than 90 percent, then regenerate/recollect at first detectable significant decline established by an appropriate statistical test.

However, it is recognized that intraspecific variation among accessions has been observed for a wide range of accessions, 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 accession sizes are often small meaning 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.

With reference to the initial seed viability testing, it is also possible that genebanks receive small quantities of seeds. In that case, it is not necessary to carry out initial seed viability testing since the sample is sent for the purpose of regeneration. However, the regenerated seeds must then be tested for viability prior to storage.

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 cryostorage 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 management plan should be activated. For example, some representative samples may need to be tested immediately following resumption of adequate storage conditions.

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4.4 Standards for regeneration

Standards

4.4.1 Regeneration should be carried when the viability drops below 85 percent of the initial viability or when the remaining seed quantity is less than what is required for three sowings of a representative population of the accession. The most-original-sample should be used to regenerate those accessions.

4.4.2 The regeneration should be carried out in such a manner that the genetic integrity of a given accession is maintained. Species-specific regeneration measures should be taken to prevent admixtures or genetic contamination arising from pollen geneflow that originated from other accessions of the same species or from other species around the regeneration fields.

4.4.3 If possible at least 50 seeds of the original and the subsequent most-original-samples should be archived in long-term storage for reference purposes.

Context

Regeneration is a key operation and an integral responsibility of any genebank that maintains orthodox seeds. It 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, which is referred to as the regeneration threshold. An accession will be regenerated when it does not have sufficient seeds for long-term storage (e.g. 1 500 seeds for a self-pollinating species and 3 000 for an out-crossing species) or when its viability has dropped below an

established minimum threshold (i.e. below 85 percent of initial viability of the stored seeds). Regeneration should also occur when the seed numbers have been depleted due to frequent use of the accession. If an accession is rarely requested and seed viability is fine, then seed numbers can be below 1 000 prior to regeneration, each regeneration of especially out-crossing species runs the risk of losing rare alleles or changing the genetic profile for the sample. Regeneration frequency should be minimized. High seed numbers are not needed for rarely requested accessions or species.

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, due attention should be paid to the environment in which the activity will be undertaken, to avoid any 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. It can often be difficult to harvest sufficient quantity of seed from wild relatives due to lower seed/plant numbers compared to other species, or plant dispersal mechanisms such as seed shattering. It is therefore necessary to ensure that appropriate technical practices are used to capture as much seed as possible (i.e. nets to capture dropped seeds).

Repeat regeneration cycles may also be required to ensure that sufficient seed is conserved. For regeneration, it is better to create favourable environmental conditions for seed production and minimize plant-to-plant competition. Conditions at the original collection sites are often unfavourable in one or more ways for maximizing seed production. So there should really be a compromise between generalized, favourable conditions and those special signals (whether photoperiodic, nutritional or climatic) that are specific to local adaptation of individual accessions. This is part of the art of curation. If the genebank site does not provide favourable conditions locally, a curator should explore means to have the collection regenerated in a favourable environment; replication of the collection environment should not necessarily be the curator's goal.

To preserve the genetic integrity of genebank collections during seed regeneration, it is important that sampling of accessions be carried out effectively. The number of seeds to be used for the regeneration process must be sufficient to be representative of the genetic diversity in an accession and to capture one or more rare alleles with a certain probability.

The methodology to be used for regeneration might vary from species to species and depends, among other factors, on the population size, breeding system and pollination efficacy. 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 (see Characterization Standards). However for cross pollinating species, it is often difficult, to use the regeneration process to carry out characterization due to logistical reasons.

Technical aspects

In order to maintain the genetic integrity of accessions it is recommended to use seeds from the most-original-sample for regeneration. For multiplication, it is recommended to use seeds from the working collection for up to five cycles of multiplication without returning to the most-original-sample.

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. For this purpose, the effective population size (N_e) 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 minimal size of N_e to minimize loss of alleles can be estimated for individual accessions based on the pollination biology and growing conditions. Best practices for harvesting should be used to avoid seed mixture during seeding, harvest and processing. Research by Johnson *et al.* (2002, 2004) on the regeneration of perennial allogamous species (e.g.

grasses) indicated that 100 plants is a minimum number which is necessary for the preservation of taxon gene pool. The principle of harvesting from 3 to 5 inflorescences from each plant is recommended.

To avoid geneflow/contamination it is critically important to use proper isolation methods between plots of accessions of cross-pollinated species being regenerated. 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 *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), 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 (Lehmann and Mansfeld, 1957). Close inspections of obtained seeds and during the first regeneration of a new genebank accession are required to collect important reference information. In order to avoid differences in seed maturity in a seed sample, multiple harvests should be carried out during the fruiting season.

Contingencies

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 for regeneration 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.

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4.5 Standards for characterization

Standards

4.5.1 Around 60 percent of accessions should be characterized within five to seven years of acquisition or during the first regeneration cycle.

4.5.2 Characterization should be based on standardized and calibrated measuring formats and characterization data follow internationally agreed descriptor lists and are made publicly available.

Context

Characterization is the description of plant germplasm. It determines the expression of highly heritable characters ranging from morphological, physiological or agronomical features to seed proteins and oil 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. It is essential that the germplasm being conserved is known and described to the maximum extent possible to assure their maximum use by plant breeders. Therefore, characterization should be carried out as soon as possible to add value to the collection. The use of a minimum set of phenotypic physiological and seed qualitative traits and morphological descriptors and information on the breeding system, such as those published by Bioversity International is helpful for characterization. Useful descriptors can also be found in the publications of the International Union for the Protection of New

Varieties of Plants (UPOV) and of the USDA's National Plant Germplasm System (NPGS). Use of internationally agreed standards for characterization data increases the usefulness of the published data.

Characterization will allow for detecting inter- and intra-accession diversity. Appropriate strategies may be necessary for ensuring the preservation of rare alleles or for improving access to defined alleles. Documentation of observations and measures taken is extremely important.

Technical aspects

Characterization is time consuming and expensive. Effort can be made to combine characterization with multiplication or regeneration to the extent possible. Curators should make all possible efforts to record characterization data. However, it is advisable to encourage the use of replication for characterization of highly heritable traits.

Characteristics and traits for crops are defined by crop experts and/or curators in consultation with genebank managers. Bioversity International has developed a wide range of crop descriptor lists for example and minimum sets of key descriptors for utilization have been established for several of these. Furthermore, there are regional and national descriptor lists available such as USDA NPGS descriptors. Data recording needs to be carried out by trained staff using calibrated and standardized measuring formats as indicated in the internationally agreed and published crop 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.

With the advances in biotechnology, molecular marker technologies and genomics are increasingly used for characterization (De Vicente *et al*., 2004), in combination with phenotypic observations because they have advantages in the estimation of uniqueness of a source of variation within or among accessions. Genotypic data obtained from characterizing germplasm using molecular techniques have the advantage over phenotypic data in that variations detected through the former are largely devoid of environmental influences (Bretting and Widrlechner, 1995). However, the use of molecular technology remains a challenge for some institutions, as it requires advanced laboratory facilities and technical capability, and could be expensive (Karp *et al*., 1997), especially in developing countries and in situations where genome-specific molecular tools e.g. simple sequence repeats (SSR) markers

have to be developed *de novo*. There are many markers and techniques available (e.g. SSR, expressed sequence tags - simple sequence repeats [EST-SSR], amplified fragment length polymorphisms [AFLP]) but, for characterization purposes, only well-established, repeatable markers such as SSR should be used. For many crops, a wide range of marker primers suitable for their use in characterization has been developed; also, minimum sets of key markers have been established. In order to ensure that the results of different analysis batches are comparable, some genebank accessions should be included as reference on each batch. The inclusion of reference accessions in molecular characterizations also plays an essential role for comparison among different genebanks.

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 that 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 that are not always available or could be costly.

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4.6 Standards for evaluation

Standards

4.6.1 Evaluation data on genebank accessions should be obtained for traits that are included in internationally agreed crop descriptor lists. They should conform to standardized and calibrated measuring formats.

4.6.2 Evaluation data should be obtained for as many accessions as practically possible, through laboratory, greenhouse and/or field analysis as may be applicable.

4.6.3 Evaluation trials should be carried out in at least three environmentally diverse locations and data collected over at least three years.

Context

Evaluation is the recording of those characteristics whose expression is often influenced by environmental factors. It involves the methodical collection of data on agronomic and quality traits through appropriately designed experimental trials. Evaluation data frequently includes insect pest resistance, plant pathology and quality evaluations (e.g. oil, protein content) and environmental traits (drought/cold tolerance and others). Adding this type of information allows more focused identification of germplasm to meet prospective client needs. Such data should then be included in the genebank's documentation system. These data sets are all highly desired by users to incorporate traits into breeding programs and improve utilization of collections. The traits for which the germplasm accessions are assayed are defined in advance by

crop experts in collaboration with gene bank curators. Reliable evaluation data that are easily retrievable by plant breeders and researchers facilitate greatly the access to, and use of, plant germplasm accessions. Germplasm may be systematically evaluated using a network approach, at international regional or national level.

Obtaining evaluation data by genebanks is time consuming and frequently more expensive than obtaining characterization data. Curators should make all possible efforts to obtain records of evaluation data. One possible source is evaluation records produced by users to whom seeds have been distributed. The genebank should solicit the user to share the evaluation data at least after a given time period that the user has published the evaluation results. Practical arrangements in this regard should be worked out between the gene bank and the recipients/users of the material.

Technical aspects

A wide range of crop descriptor lists have been developed for example by the International Board for Plant Genetic Resources Institute (now Bioversity International) and UPOV. Furthermore, there are evaluation descriptor lists developed by regional and national organizations such as USDA-ARS NPGS descriptors.

Data collection should be conducted by trained staff using as much as possible calibrated and standardized measuring formats with sufficiently identified check accessions (controls) and published crop descriptor lists. The results of greenhouse, laboratory or field evaluations, following standardized protocols and experimental procedures are usually presented as either discrete values (e.g. scores for severity of disease symptoms; counting) or continuous values (based on measuring). The data need to be validated by curators and documentation officers before being uploaded into the genebank database and made publicly available. The participation of multi-disciplinary teams with expertise in seed biology and plant pathology, pest resistance, environmental tolerances, both in-house and from collaborating institutes, during the process of evaluation is desirable. Often these requirements are unlikely to be met by genebanks and evaluation of germplasm accessions is best together with specialist plant breeders.

Many agronomic traits required by breeders are too genetically complex to be screened for in the preliminary evaluation of germplasm accessions. Data on agronomic traits are usually obtained during the evaluation of germplasm in a breeding program, and many of these traits result from strong genotype by environment (G × E) interactions and hence are site-specific. It is essential to use replications for the evaluation of desired traits in different environments and to

clearly define and identify check accessions to be used over the years. This should be carried out in at least three environmentally diverse locations and over three vegetative cycles and data compared across years in a statistically sound manner.

With the advances in biotechnology, molecular marker technologies and genomics are increasingly used for evaluation as well (De Vicente *et al*., 2004) (see Standards on characterization). The most commonly used molecular markers in germplasm characterization and evaluation include AFLPs, SSRs, and single nucleotide polymorphisms (SNP). They have largely replaced the older marker types, restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) on account of their relative genomic abundance and the high reproducibility

of data. In addition, advances in next generation sequencing and the accompanying reduction in costs have resulted in the increasing use of sequencing-based assays such as the sequencing of coding and non-coding regions and genotyping-by-sequencing (GBS) in germplasm evaluation. Molecular markers vary in the way they detect genetic differences, in the type of data they generate, in the taxonomic levels at which they can be most appropriately applied, and in their technical and financial requirements (Lidder and Sonnino, 2011). Where marker assisted selection (MAS), i.e. the selection for the presence or absence of traits in breeding materials at the molecular level, is feasible, it can also be applied in the evaluation of germplasm for traits of interest. The dearth of adequately skilled personnel and the lack of resources for the relatively high set-up costs continue to prevent the widespread adoption of molecular markers as a method of choice for germplasm evaluation especially in developing countries.

Contingencies

The evaluation of plant germplasm is very labour-intensive and requires adequate levels of sustainable funding to allow for the assemblage of reliable high quality data. In situations where carrying out the full evaluation of all accessions, which though desirable may not be economically feasible, the selection of genetically diverse accessions (based for instance on previously delineated sub-sets of germplasm collections) is recommended as a starting point.

Variations in the incidences of pests and diseases, the severity of abiotic stresses and the fluctuations in environmental and climatic factors in the field impact on the accuracy of data and should be mitigated through reasonably replicated, multi-locational, multi-season and multi-year evaluations. In addition, the laboratory assays for the measurements of some traits like oil or protein contents, starch quality, nutritional factors, etc. require specialized equipment that are not always available or could be costly, underscoring again the need for the participation of multi-disciplinary teams from several organizational units or institutions as the case may be.

Using the evaluation data generated by others could pose significant practical challenges. For instance, the data may be in different formats, and if published already may involve copy right and intellectual property rights issues. In order to facilitate the use of externally sourced data, it is therefore important to standardize data collection and analysis, and provide uniform reporting formats.

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4.7 Standards for documentation

Standards

4.7.1 **Passport data of 100 percent of the accessions should be documented using FAO/Bioversity multi-crop passport descriptors.**

4.7.2 **All data and information generated in the genebank relating to all aspects of conservation and use of the material should be recorded in a suitably designed database.**

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 FAO/Bioversity multi-crop passport descriptors (Alercia *et al.*, 2012) 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 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. It is important to put in place a backup of the database system off-site.

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

With advances in biotechnology, there is a need to complement phenotypic trait data with molecular data. Efforts must be made to record the molecular data being generated through genomics, proteomics and bioinformatics.

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/Bioversity List of Multi-crop Passport Descriptors (Alercia *et al.* 2012) 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, GENESYS, Mansfield Database (IPK) and SESTO (NordGen),[1] which have been specifically developed for genebanks and their documentation and information management needs. Another germplasm information management system is the International Crop Information System (ICIS) platform in which germplasm data from 1 or more genebanks can be stored, and published online with a search-query capacity to allow users to set criteria for selection of germplasm by single or by multiple trait criteria, as well as bounded by GPS coordinates for a region and/or overlaid with climatic and soil maps, for targeted selection of germplasm.

1 GRIN: http://www.ars-grin.gov.
GENESYS: http://www.genesys-pgr.org.
Mansfield Database: http://mansfeld.ipk-gatersleben.de/pls/htmldb_pgrc/f?p=185:3:1644539197326401.
SESTO: http://www.nordgen.org/sesto.

Evaluation data are 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. However, it is recognized that using information generated by users may not be so simple and may involve copyright and institutional issues.

Contingencies

Lack of documentation or loss of it compromises the optimal use of the seeds or can even lead to their loss. Curators should ensure to maintain proper records of all information associated with genebank management in backup documentation systems, as part of their risk management system. In the event where no computer-based system is available, all-important information should properly be documented in ledgers.

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4.8 Standards for distribution and exchange

Standards

4.8.1 Seeds should be distributed in compliance with national laws and relevant international treaties and conventions.

4.8.2 Seed samples should be provided with all relevant documents required by recipient country.

4.8.3 The time span between receipt of a request for seeds and the dispatch of the seeds should be kept to a minimum.

4.8.4 For most species, a sample of a minimum of 30–50 viable seeds should be supplied for accessions with sufficient seeds in stock. For accessions with too little seed at the time of request and in the absence of a suitable alternative accession, samples should be supplied after regeneration/ multiplication, based on a renewed request. For some species and some research uses, smaller numbers of seeds should be an acceptable distribution sample size.

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. There is a continuous increase in demand for genetic resources to meet the challenges posed by climate change, by changes in virulence spectra of major pests and diseases and by invasive alien species. 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. When a user requests an accession from a genebank, the user is responsible for indicating the national requirement for seed importation, in particular the phytosanitary regulations, in their country in order to avoid the spread of quarantine or regulated pests or invasive species that could seriously affect national production.

The two international instruments that govern the access of genetic resources are the ITPGRFA and the CBD. The ITPGRFA facilitates access to PGRFA, and provides for the sharing of benefits arising from their utilization. It has established a multilateral system for PGRFA for a pool of 64 food and forage crops (commonly referred to as Annex 1 crops to the Treaty), which are accompanied by an SMTA for distribution. SMTA can also be used for non-Annex 1 crops; however, other models are also available. Access and benefit sharing under CBD is according to its Nagoya Protocol. Both the ITPGRFA and CBD emphasize the continuum between conservation and sustainable utilization, along with facilitated access and equitable sharing of benefits arising from use.

Genebanks should aim at making available to users as many accessions as possible including associated data. When stock is depleted, the accessions should be multiplied to meet the demands of users as a matter of priority. Genebanks should promote the availability of genetic resources for uses including research, breeding, education, farming and repatriation. Internationally, genebanks can be a source of landrace germplasm to re-supply countries which are initiating their

own genebank, or which suffered a disaster such as fire, flood or civil strife. It is to be noted that the minimum number of seeds to distribute is species dependent and usage dependent. Genebank accessions are not only used for pre-breeding and applied plant breeding, but also for research activities. In the latter case, often very few seeds are needed.

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 regulations and laws. The elements of national regulations and laws, in particular seed health requirement has to be provided by the user or the national phytosanitary authorities.

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, additional declarations, certificate of donation, certificate of no commercial value and import permit and others 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. If additional costs (phytosanitary certificates, ISTA bulletin, specific envelopes or other) are necessary for the seed distribution or exchange, these costs have to be at the charge of the user, or otherwise determined by both parties. A major problem with international distributions is that genebanks have to declare that a particular disease was not found in the seed production field. Genebanks cannot meet additional declaration requirements for seed that was produced 20–30 years ago. Countries that receive seed should be responsible for quarantine procedures to handle seed where additional declaration requirements cannot be met.

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 should 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.

Genebank accession recipients are encouraged to do their own seed bulking for their trials needs and experiments. This is particularly relevant for wild species for which seed stock are often low and for replicated field trials where supply of the required seed quantity cannot be considered.

For material distributed outside the multilateral system of the Treaty, the distributing genebank should encourage the flow back of information about the usefulness of the supplied germplasm from the recipient to the provider according to the terms of the MTA.

Contingencies

Political decisions, crisis situations or bureaucratic delays 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 and delay the distribution process.

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4.9 Standards for safety duplication

Standards

4.9.1 A safety duplicate sample for every original accession should be stored in a geographically distant area, under the same or better conditions than those in the original genebank.

4.9.2 Each safety duplicate sample should be accompanied by relevant associated information.

Context

Safety duplication assures the availability 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 long-term 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, including database backup. The safety duplication of the materials is deposited in long-term storage at a different location. The location is chosen to minimize possible risks and provides the best possible storage facilities. To minimize risks that can arise in any individual country safety duplication will be ideally undertaken outside that country.

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. It is recognized however that the black-box is not the only approach. There may be cases where the safety collection is also taken care of by the recipient genebank.

Safety duplication should be made for all original seeds collected by the genebank or when only held by the genebank. However, the genebank should retain a set of the original samples to facilitate access for regeneration or other managerial decisions. Seeds that 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.

Safety duplication is available at the Svalbard Global Seed Vault on Spitsbergen Island, Norway. Institutions depositing seeds retain ownership and access to samples stored in Svalbard is granted to the depositor only.

Technical aspects

When selecting the location for safety duplication, primary consideration is given to the geographic location and environmental conditions of the location. Facilities 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. 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. In some cases, it is helpful to sort material according to short, medium and long living seed groups before sending for safety duplication.

Sample size should not be restricted to a certain minimum number. Sample size should be sufficient to conduct at least three regenerations. A safety backup is not just for future regeneration; it may also provide a minimum sample to regenerate an accession that was lost. A "critical" safety backup with a minimal amount of seed at a second location is better than no backup at all. If possible, 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 for safety duplication should be of trilaminate material of which the middle metal foil layer should be of adequate thickness. It should be formed into a pouch seamed on all four sides with no gusset. This would provide an adequate water barrier for transport and storage at 18 °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 (cardboards 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 long-term collection of the sender starts to decline.

Contingencies

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.

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4.10 Standards for security and personnel

Standards

4.10.1 A genebank should have a risk management strategy in place that includes *inter alia* measures against power cut, fire, flooding and earthquakes.

4.10.2 A genebank should follow the local Occupational Safety and Health requirements and protocols where applicable.

4.10.3 A genebank should employ the requisite staff to fulfil all the routine responsibilities to ensure that the genebank can acquire, conserve and distribute germplasm according to the standards.

Context

Achieving a genebank's goal of acquisition, conservation and distribution of germplasm not only require 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 complements 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 backup; 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.

Technical aspects

Staff should have adequate training acquired through certified training and/or on-the-job training and training needs should be analyzed. 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 backup 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 rooms 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 necessary 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 periodic checks should be made on the electrical circuitry to ensure compliance with safety standards. Fire-fighting equipment will include extinguishers and fire blankets. For areas affected by thunderstorms, a lightning rod should be fitted to the genebank.

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 or to approach other genebanks for assistance. The international community of genebanks should be informed, if the functions of the genebank are endangered.

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.

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