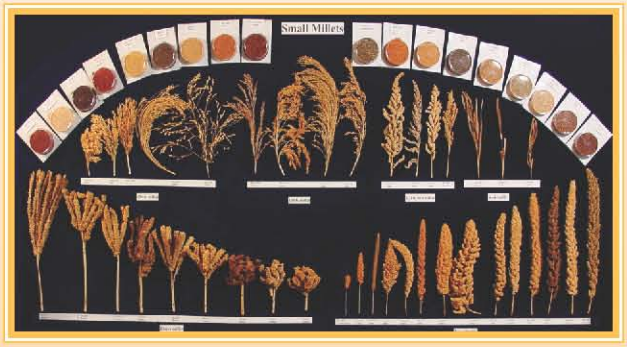
Technical Manual no. 10

Managing and Enhancing the Use of Germplasm – Strategies and Methodologies



ICRISAT
Science with a human face

International Crops Research Institute for the Semi-Arid Tropics

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Managing and Enhancing the Use of Germplasm – Strategies and Methodologies

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Figure 4C.1.1. Seed-drying cabinet at ICRISAT genebank.

Figure 4C.1.2. Walk-in seed drying room at ICRISAT genebank.

The drying cabinet should have safety devices to regulate the temperature and prevent overheating in the event of failure of thermostat.

Prediction of drying period by weight loss

- Determine the moisture content of the seed sample using the methods described in Section 4B.
- Weigh the seed sample that requires drying.
- Calculate the weight of the seeds at required moisture content by the equation:

$$\text{Final seed weight} = \text{Initial weight of seeds} \times \frac{(100 - \text{Initial \% moisture content})}{(100 - \text{Final \% moisture content})}$$

- Keep the sample in a muslin cloth bag and allow it to dry until the required weight is attained.

If there is no previous experience of drying seeds of particular species, it may be necessary to do some experimental work to predict the appropriate drying period.

Prediction of drying period from mean drying curves

- Determine the moisture content of the seed lots using methods described in Section 4B.
- Keep the seed lots in labeled muslin cloth bags and place them in drying environment.
- Remove a small sample and repeat moisture determination of the seed lot every day.
- Plot the moisture content of the seeds on a graph with percentage moisture content on Y-axis and drying time on X-axis.

Seeds dry at an exponential rate until equilibrium moisture content is reached. The rate of drying of different seed lots of the same species will be more or less similar.

The drying curves under a constant drying environment of 15°C and 15% RH for seeds of sorghum, pearl millet, chickpea, pigeonpea and groundnut are shown below (Fig. 4C.1.3).

The following graphs can be used for predicting the drying period of all seed lots of a particular species dried under similar conditions.

- Draw a horizontal line each from the initial and desired moisture contents on the Y-axis across to the drying curve.
- Mark or read the day on X-axis for the two points of intersection.

The difference between the two points gives the drying time required to achieve the desired moisture content.

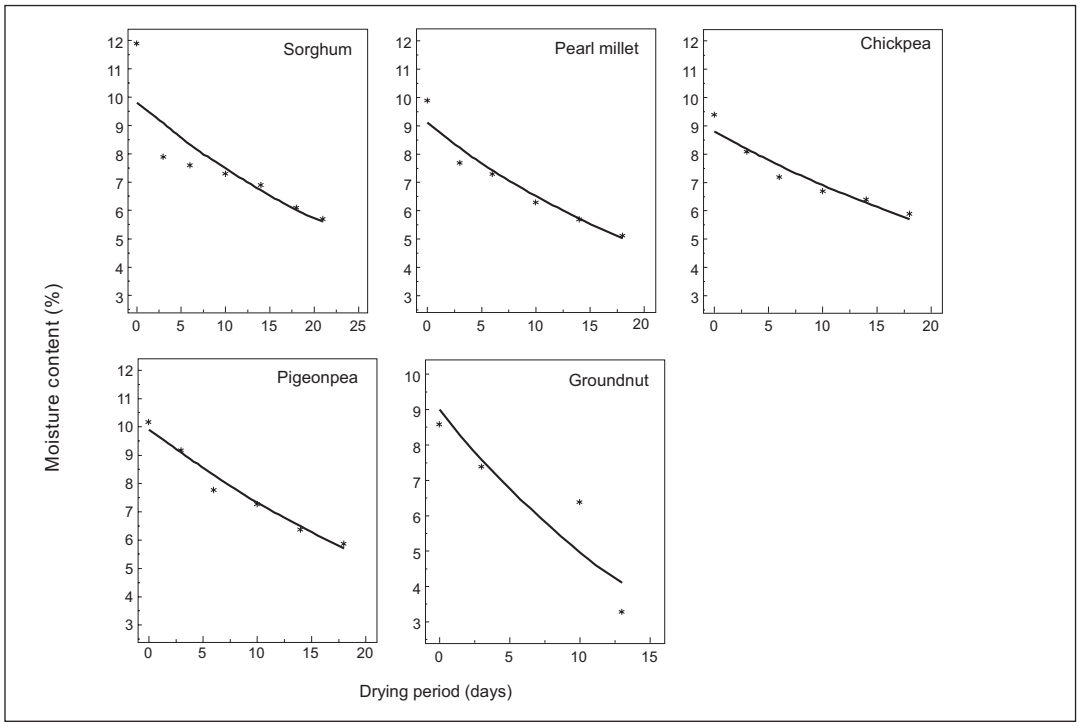


Figure 4C.1.3. Seed drying curves under a constant drying environment of 15°C and 15% RH for seeds of ICRISAT mandate crops.

4C.2. Silica gel drying

Small samples can be dried using silica gel (see Fig. 4C.2.1).

- Place dried silica gel (deep blue in color) in desiccators or glass jars with an airtight seal. The weight of the silica gel used should be equal to the seeds for efficient drying.
- Place the seeds in muslin bags and keep them in close proximity to the silica gel.
- Keep the desiccator at 15°–20°C.
- Change the silica gel daily or when the color changes from deep blue to pink or pale blue.
- Regenerate the silica gel by heating at 100°C until it turns deep blue again and allow it to cool in an airtight container for reuse.
- Leave the seeds with fresh changes of silica gel in the container until the moisture content of the seeds is in the range required for storage.
  - ➢ if the initial moisture content and weight of seed lot are known, the weight of seeds at required moisture content can be calculated by the weight loss using the following equation:

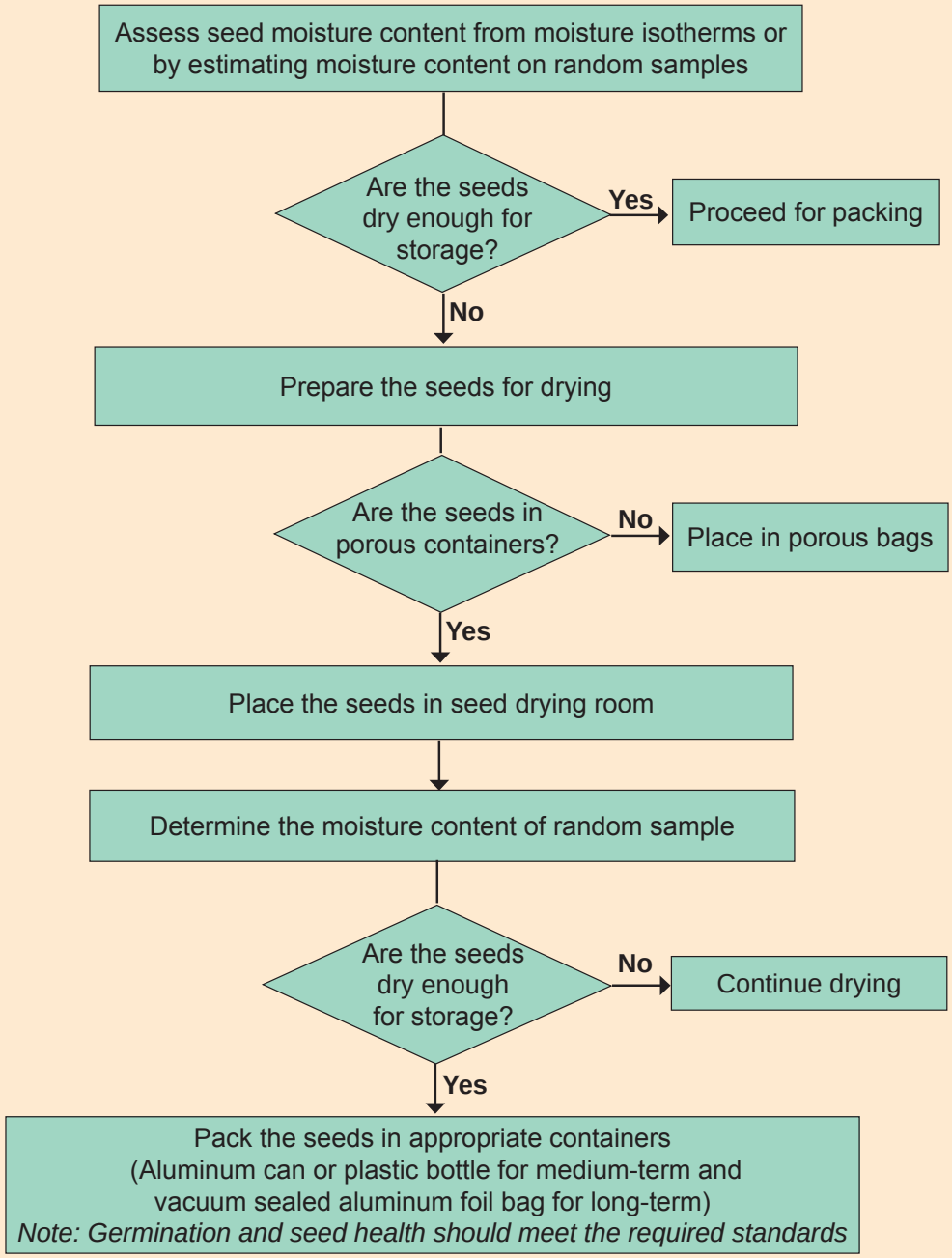
$$\text{Final weight} = \frac{\text{Initial weight} \times 100 - \text{Initial moisture content (\%)}}{100 - \text{Final moisture content (\%)}}$$

  - ➢ alternatively, remove a subsample and determine whether or not the required moisture content is attained, using methods described in Section 4B.

- Pack the seeds in appropriate containers once the recommended moisture content or the equilibrium seed weight is attained and if the germination and seed health are acceptable.
- If the moisture content is not low enough for storage, continue further drying.

Figure 4C.2.1. Seed drying using silica gel at ICRISAT genebank.

Seed drying – Flow chart



4D. Seed viability testing

Viability tests measure how many seeds germinate and develop into plants, which reproduce themselves.

- Viability of accessions should be tested:
  - before seeds are packaged and placed in the genebank, and
  - at regular intervals during storage.

Many methods are available to test seed-viability. The most accurate method to test seed viability is the germination test using appropriate procedure.

4D.1. Germination test

Complete germination can be achieved only under optimum conditions of light, temperature and water. The requirements for germination vary with species as shown in Table.4D.1.1.

Table 4D.1.1. Recommended conditions for germinating seeds of ICRISAT mandate crops.

Crop	Substrate*	Temperature	Special requirements**
Sorghum	BP	20/30°C (16/8h); 20°C	0.2% KNO_3 for wild species
Pearl millet	TP	20/30°C (16/8h); 20°C	
Chickpea	BP	20°C	Mechanical scarification for wild species
Pigeonpea	BP	25°C	Mechanical scarification for wild species
Groundnut	BP	25°C	Remove shell, 0.2% ethrel***
Finger millet	TP	20/30°C (16/8h)	
Foxtail millet	TP	20/30°C (16/8h)	
Little millet	TP	Not available	
Proso millet	TP	20/30°C (16/8h)	Light 180 × 10^{-6} m^{-2} s^{-1}, 12 h/d
Barnyard millet	TP	20/30°C (16/8h)	Prechill, light
Kodo millet	TP	20/30°C (16/8h)	

* TP = Top of Paper, BP = Between Paper (Paper towel method)

** Freshly harvested seeds and wild species of most crops show dormancy, ie, the seeds remain hard and firm during the germination test. Special treatments are required to overcome dormancy.

*** Prepared by diluting 2 mL ethrel (2-chloroethylphosphonic acid) with 998 ml distilled water.

Sample size

- Use a minimum of two replicates each of 50 or 100 seeds for testing initial germination and two replicates each of 25 or 50 seeds for subsequent tests, depending on available seed quantity.
- Take a random sample of seeds from the container.
- If the seeds are very dry (moisture content <8%) expose them to ambient atmosphere for 24 h to raise the moisture content before testing for germination.

Seed collected in the field should be quickly processed, packaged in appropriate containers and stored as soon as possible.

5A. Medium- and long-term conservation

Maintaining genetic integrity, which is the main priority of a genebank curator, can be achieved by storing the original seeds (or from initial multiplication) as ***base collections*** under long-term conditions in sufficient quantity.

If the genebank has distribution of germplasm as a function, adopt a two-step storage system and maintain ***active collections*** of the sample under medium-term conditions.

If the genebank has distribution as a function, adopt a two-step seed storage system to avoid repeated regeneration. In this system, two separate seed samples are maintained - one for multiplication (Base collection) and the other for distribution (Active or Working collection).

- ***Base collections are stored under better storage conditions (long-term, -20°C) than those for Active collections (medium-term, 4°C and 20-30% RH).***
- ***Demand for seeds are met by distributing the samples from the medium-term store until the seed is about to be exhausted or the seed viability begins to decline. Then some seeds are taken out from long-term store and given for multiplication. The multiplied seeds are stored in medium-term for further distribution.***
- ***These cycles are repeated until the seeds in long-term are about to be exhausted or the viability of seeds has declined.***
- ***When the seeds in long-term storage are due for regeneration, fresh seeds produced by multiplication are used both to replenish seed stock in medium-term and storage in long-term.***

The number of regenerations through which seeds are passed before distribution is thus reduced. Consequently, high quality seeds with minimal genetic change are made available for distribution, while original or near original seeds are available for regeneration.

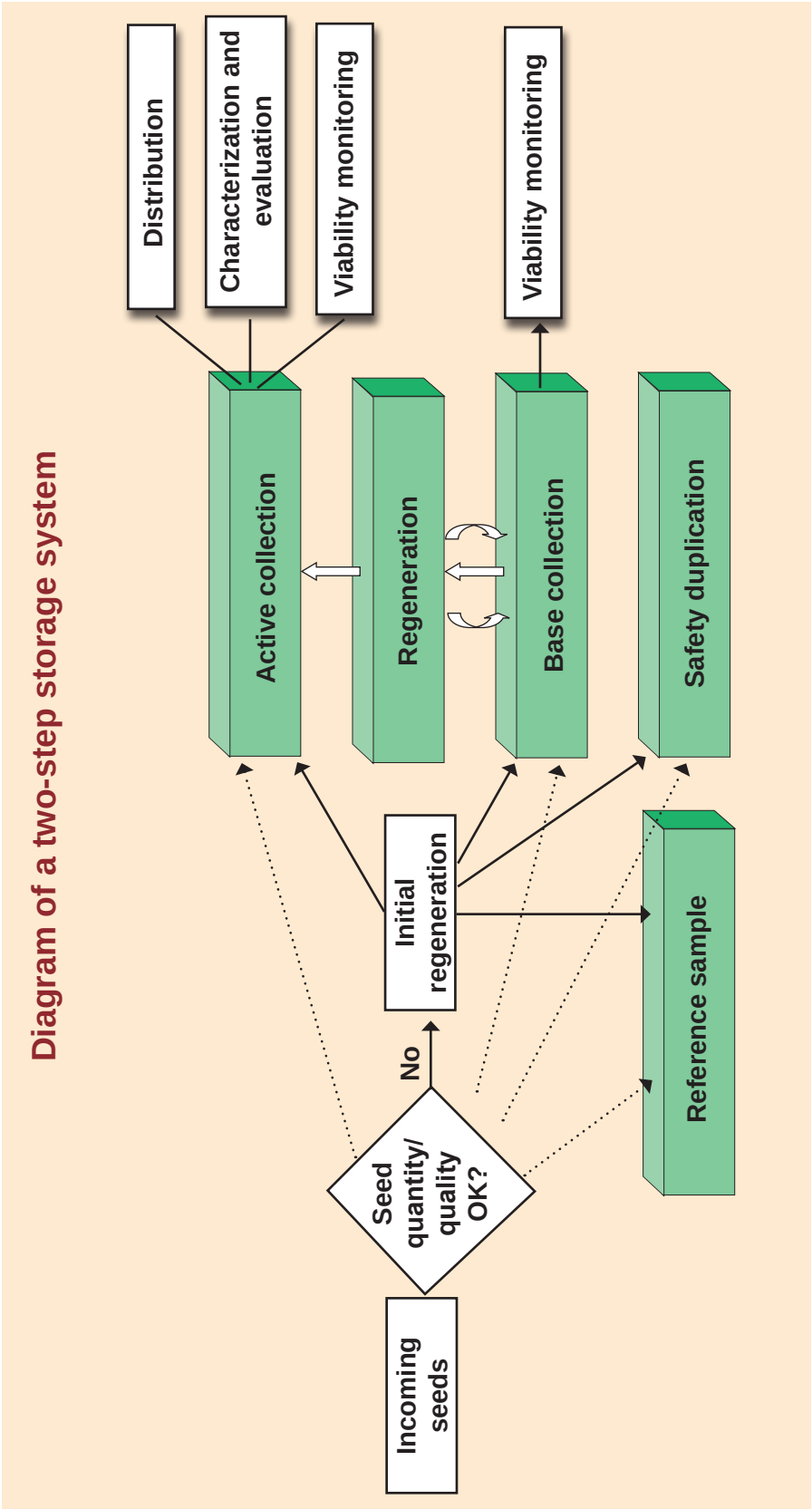


Diagram of a two-step storage system
Distribution
Characterization and evaluation
Viability monitoring
Viability monitoring
Active collection
Regeneration
Base collection
Safety duplication
Initial regeneration
Reference sample
No
Seed quantity/ quality OK?
Incoming seeds

5A.1. Base collection

Base collection is a set of accessions preserved for long-term future. Each accession is distinct, and in terms of genetic integrity is as close as possible to the sample originally collected or acquired.

Seeds ***are not*** distributed from the base collection.

- Preferred storage conditions are:
  - -20°C with 3–7% seed moisture content, depending on species.
- Acceptable storage conditions are:
  - sub-zero temperature with 3–7% moisture content.

Accession size

- At least 1,000 viable seeds, but preferably 1,500–2,000 seeds should be stored for materials showing little morphological variation (genetically homogeneous accessions) as with chickpea and groundnut.
- For materials showing large morphological variation (genetically heterogeneous accessions) the accession should consist of at least 4,000 seeds, but preferably 12,000 seeds as with sorghum, pearl millet and pigeonpea.

Seed viability

- Seed placed in base collection should have >85% germination in groundnut and >90% in other crops. The minimum germination standard for wild species is 75%.

Seeds in base collection are not used for distribution. They are used only for regeneration.

5A.2. Active collection

Active collection comprises accessions that are available for immediate multiplication, distribution and use. Since these accessions are accessed frequently, they are maintained under medium-term conditions, which ensure that accession viability remains above at least 65% for 10–20 years. A combination of storage temperature and moisture content is given below:

	Moisture content (%)	
Temperature (°C)	Groundnut	Sorghum, millets, chickpea, pigeonpea
25	2.0	6.5
20	3.5	7.5
15	5.0	8.0
10	6.0	9.0
5	7.0	10.0
0	8.0	11.0

Active collections at ICRISAT genebank are maintained at 4°C and 20–30% RH.

Accession size

Accession size depends on the demand for the accessions. Frequently requested materials can be stored in larger quantities than others. The maximum sample size held in active collections at ICRISAT genebank is given below:

- Sorghum, pearl millet, chickpea, pigeonpea: 400 g.
- Groundnut: 1.5 kg.

The weight of seeds can be converted into seed number using the 100-seed weight. For example,

if 100-seed weight is 2.5 g,

400 g contain: 100 × 400/2.5 = 16,000 seeds

Seed viability

Seeds placed in the active collection should have >80% germination in groundnut and >85% in other crops.

Location in storage

The physical location of the accession in the genebank should be coded to locate it easily for retrieval of seeds, etc. The location of an accession in the ICRISAT genebank is coded as follows:

- Room: 1–7.
- Rack: A–Z.
- Bay: I–IV.
- Tray: 1–999.

For example, the code 3-B-IV-12 indicates the location of sample as Tray no. 12 in Room 3, Rack B and Bay IV.

Assigning location code

- Check the inventory data file to find the next available space for the accession.
- Assign the space where the accession is to be placed.
- If the accession is stored in more than one container, keep them all together.
- Place the container in seed store in the assigned location.
- Enter the details (location, date of storage and number of containers) in the inventory data file.

5B. Safety duplication

Safety duplication means a genetically identical duplicate accession sample stored outside the country in a base collection for safety reasons. Safety duplication ensures that any given collection is securely duplicated at another institute, which can preserve the material safely. This provides insurance against loss of material. Under the Agreement with FAO (on behalf of ITPGRFA General Body), ICRISAT has the responsibility of making arrangements for the duplication of its collections. Safety duplication includes both the duplication of the material and the documentation process.

Types of duplication include:

- ***Black box*** — when the responsibility of the recipient's institute is to maintain the duplicates in adequate storage facilities without handling the samples. It is the originator's responsibility to monitor seed viability and, when necessary, regenerate the collection.

For black box duplication, special permissions are required to export the seeds without Phytosanitary Certification from the originating country. Similarly, the Plant Quarantine Authority in the destination country needs to permit the importation of seeds by the recipient, bypassing the routine quarantine examination.

Prepare the samples for safety duplication similar to the base collections:

- Seeds should be dried to moisture content 5 ± 2%.
- Seeds should be clean and healthy.
- Percent germination should be >85%.
- Vacuum sealed in laminated aluminum foil packets.
- The minimum sample size can be small, ie, approximately 25 g for sorghum and millets, and 100 g for legumes (Fig. 5B.1).
- To save time, the samples for safety duplication can be set aside at the time when the seeds for long-term conservation are processed.

Figure 5B.1. Germplasm samples packed for safety duplication at Svalbard Global Seed Vault, Norway.

5C. Storage policy of ICRISAT genebank

All FAO/ITPGRFA designated germplasm and newly acquired material, which is threatened and of value, will be conserved.

The following are conserved as base collection:

- Germplasm currently designated for FAO /ITPGRFA
- All landrace accessions collected or acquired in the future with complete passport information

ICRISAT Plant Material Identification Committee (PMIC) released the best of the breeding material received with complete pedigree information and key characterization data.

The medium-term storage conserves working collections of:

- Frequently distributed material
- Core and mini core collections
- Genetic stocks
- Undesignated stable breeding lines
- Wild species
- Emergency national holdings.

5D. Documenting inventory data

The genebank should maintain proper documentation to allow rapid accessioning of new samples, answer queries on the conserved germplasm and monitor quality and quantity of stored material to carry out regeneration and distribution. A computerized data handling system is ideal for a genebank. The genebank inventory data includes details of accessions held in storage, their location, quantity and quality. The suggested descriptors are:

ICRISAT accession identifier: Unique identifier for accession assigned when the sample is entered into the collection.

Season of harvest: Season when the crop was harvested (mm/yy).

Site of rejuvenation: Place where the accession was regenerated.

Container: Type of container used for storage, eg, plastic bottle, aluminum can and aluminum foil packet.

Number of containers: Total number of containers used for storing the sample.

Date of storage: Date on which the sample was placed in genebank.

Location in genebank: Exact location where the sample is stored in the genebank — coded for example, 05-A-VI-12, indicating room, rack, bay and tray numbers.

Seed quantity (g): Quantity of seeds currently available in storage.

Germination (%): Percent seed germination from the result of the recent germination test conducted.

Date of germination testing: Date on which seeds were tested for germination.

Remarks: Any significant observation.