Processing of Orthodox Seeds for Long Term Conservation in National Genebank

Chithra Devi Pandey and Padmavati G. Gore
Division of Germplasm Conservation, ICAR-NBPGR, New Delhi

Conservation of plant genetic resources is of high priority in the national context, as a Global Plan of Action (GPA) activity or as requirement of Convention on Biological Diversity (CBD). The National Plant Genetic Resources programme in India has already developed elaborate guidelines for Long Term Conservation of germplasm in National Genebank, which maintains international standards and also ensures long term viability of the germplasm conserved after due processing. The seeds are best stored at sub-zero temperature with 3-7 percent moisture content (IPGRI, 1994) depending on species.

The following parameters are check listed before sending the material for *ex- situ* conservation

i. Well-developed and physiologically mature seeds.
ii. Free from insects, weeds and disease.
iii. Clean and free from under sized, shrivelled, immature and discolour seeds.
iv. Properly labelled and packed to avoid damage during transit.
v. Untreated with chemicals.
vi. Send to gene bank at the earliest possible (immediately after harvest).
vii. Accompanied with the passport data

The operational sequence (Fig 1) to integrate an accession into the genebank involves different activities, which are being performed by different divisions/units as detailed below:

Unit/ Division involved in Processing	**Activities**
Germplasm Handling Unit (Division of Germplasm Conservation)	• Receipt of the sample and Sample verification/registration
Division of Plant Quarantine	• Seed health testing
Division of Germplasm Conservation	• Seed viability test/checking the germination percentage • Seed moisture estimation • Seed drying • Seed packaging • Long Term Storage

Germplasm Handling Unit (GHU)

a) Verification of seed samples received in corresponds with the accompanying list.
b) Check whether the sample is already present in the genebank(to avoid conserve ring duplicates)
c) Check the condition of the seeds (e.g. damage by the insect, fungal growth, damaged, broken, empty or shriveled seeds).
d) Cleaning of the sample
e) Sent to Plant Quarantine Division for seed health testing

Division of Plant Quarantine (DPQ)

a) Seed health testing
b) Assuring pest free conservation through quarantine examination

Division of Germplasm Conservation (DGC)

a) Seed Cleaning
b) Initial Viability Testing.
c) Seed Quantity Determination

d) Moisture Determination
e) Seed Drying
f) Seed Packaging
g) Long Term Storage (Base collection)
h) Documentation

a) Seed Cleaning

Seed cleaning is the removal of debris, inert material, damaged and infested or infected seeds and seed of different species (e.g., weeds) to achieve clean and pure samples of seeds of high physiological quality for storage. Seed cleaning is necessary to:

- Improve purity of the sample.
- Optimize storage space and reduce costs.
- Protect seeds from fungal infection during storage and initial field growth.
- Allow precise regulation of seed moisture content during storage.

Seeds should be cleaned immediately after harvest or soon after, they arrive at the genebank. Cleaning methods vary according to the type of seed.

b) Initial viability testing

The initial seed viability test should be conducted after cleaning the accession. If viability test is delayed due to unavoidable reason, the seed samples should be temporarily kept in moisture pervious containers like muslin cloth bag in a walk-in drying room maintained at 15-20% RH and 15^0C temperature where the moisture content will get equilibrated to 5-7% depending on the crop species. The initial germination value should more than 85 per cent for most of the cultivated species of Agricultural crops. Exceptions may be granted for some specific accessions of horticultural crops, forestry species, forage grasses and crop wild relatives where quality seed production problems exist, by taking into consideration their reproductive biology, basic seed characteristics, seed multiplication ratio and growth cycle. The Indian Minimum Seed Certification Standards for viability, wherever available, may be acceptable for long-term storage.

Germination testing methods:

Top of paper (TP): The seeds are germinated on top of one or more layers of paper which are placed into transparent Petri dishes. The appropriate quantity of water is added at the beginning of the test and evaporation may be minimized by a tightly fitting lid.

Between paper (BP): The seeds are germinated between two layers of paper, loosely covering the seeds with an additional layer of filter paper and then placing the seeds into folded envelopes, which may be placed in a upright position in a cabinet germinator, provided the relative humidity in the germinator can be maintained very near saturation.

Pleated paper (PP): The seeds are placed in a pleated, accordion-like paper strip with 50 pleats, usually two to a pleat. The pleated strips are kept in boxes or directly in a 'wet' cabinet, with a flat strip often wrapped around the pleated paper to ensure uniform moisture conditions. This method may be used as an a alternative where TP or BP are prescribed.

c) Seed Quantity Determination

Several methods are used to estimate the number of seeds within accessions. The determination and method is recorded on the Seed Bank Database, which, where appropriate to calculate the final seed quantity. The method used to estimate quantity depends on a number of factors:

1) If there are only a few seeds (less than about 300), they are counted individually.
2) A collection may be counted entirely by a seed counting machine if it is clean and has suitably sized seeds. However, this method is too slow for very large collections.


Cabinet germinator


Evaluation of germination test

d) Moisture determination

Moisture content of the seed is the most critical factor, which controls the keeping quality of seeds. At the time of harvest, seeds have a higher range of moisture *viz* 12-16% based on crops, and agro-climate. Such high moisture contents are detrimental to seed viability. Although the FAO/IBPGR recommendation for seed moisture content at the time of storage is 3-7%, it is suggested that for edible oil seeds as well as seeds with higher oil content the range should be 3-5% and for rest of the field and forestry species with known orthodox behavior, it should be 5-7%.

Seed Moisture Content (SMC) can be determined by two different methods

- The oven-drying method, described by ISTA (2015); and
- Moisture meters.

ISTA (2015) has prescribed two different oven-drying methods for determining moisture content, based on the chemical composition of seeds;

- The low constant temperature oven method for oily seeds; and
- The high constant temperature oven method for non-oily seeds.

Pre-drying of the seeds is must if the initial moisture content is suspected to be above 17%. For moisture content determination the seed weight should be 0.5-1 gram or a minimum of 10 seeds in three independent replicates. .Some seeds require grinding into smaller particles to promote uniform and complete drying Some of the common species which require grinding are barley, groundnut, cotton, chickpea, *citrullus*, oat, sorghum .For oily seeds the low constant temperature oven method is used i.e. 103^{O}C for 17hrs. For non-oily seeds, the moisture content is determined by high constant temperature oven method i.e. 130^{O}C for one hour (ISTA 2015).

SMC is expressed in terms of the weight of water contained in seed as a percentage of the total weight of the seed before drying, known as the wet-weight (wb) or fresh-weight basis.

SMC (% wb) = wet weight – dry weight /wet weight x 100

Moisture content can also be expressed on a dry-weight basis (db)-that is, the loss in weight as a percentage if the dry weight of the seeds.

SMC (% db) = wet weight – dry weight /dry weight x 100

e) Seed Drying

Dehumidifier dryer

Specially designed seed dryers, which can maintain 15% RH and 15^{0}C temperature is used to allow slow and safe drying, where minimum loss of seed quality occurs. The most common and safe method of drying of seeds used in gene banks are dehumidifier dryer. The following precautions need to be taken while drying the orthodox seeds.

- Place each accession in labeled paper envelops or cloth bags.
- One accession can be split into several labeled bags to help rapid drying.

- Do not stack the bags too closely and use open racks in a drying room or cabinet with a fan to allow air to circulate.
- Leave the seeds in the drying area until the moisture content is predicted to be in the range 3-7% required for storage.

NGB-NBPGR: Walk-in seed drying room

NGB-NBPGR: Seed drying cabinet

Desiccator dryer

- Small seed quantity/released variety/wild/weedy type can be dried with the use of silica gel in an enclosed space such as a desiccators, can or glass jar with an airtight seal.
- Fresh deep blue silica gel used for rapid drying of the seeds.
- Containers are placed in the a room held at approximately 15-20°C when the seeds are drying.
- Silica gel is changed daily or when the color changes from deep blue to pink.

Cabinet Desiccators

Silica Gel Drying

Silica Gel

f) Seed Packaging

Packing of seed material is essential to prevent absorption of water from atmosphere after drying, to keep accessions separate and to prevent contamination of seeds from insects and diseases.

Factors influencing seed packing

- Kind of seed to be packed
- Duration of storage
- Storage environment
- Seed moisture content
- Cost of packing material
- Geographical area where the seed will be stored

Choice of packing material

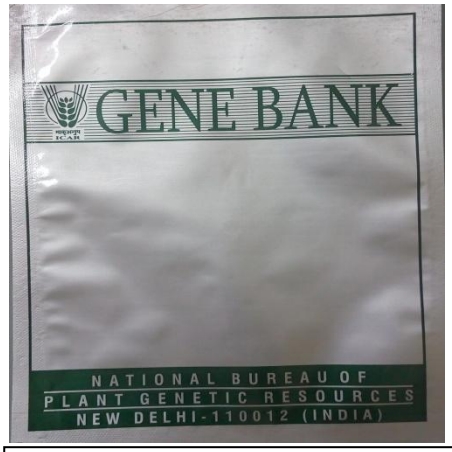
- **Moisture vapour permeable Containers-**
  Jute bag, cloth bag, paper bag, multiple paper bags etc.
- **Moisture vapour resistant container**
  Jute bag, laminated with 200 to 300 gauge, polyethylene film.

- **Moisture vapour proof containers**
  Tin cans, polyethylene bags (over 700 gauge thickness) and Tri-layered Laminated Aluminum Foil (LAF) pouches which have 12 micron outermost layer of polyester, 12 micron middle layer of aluminum foil and 250 gauge inner layer of polyethylene.

Of the above mentioned moisture proof containers, tri-layered aluminium foil pouches are the most preferred ones as

- They are easy to pack and can be cut to desire size.
- It saves space
- Can be sealed again after use and are
- Capable of withstanding temperature of -20^0C to 40^0C

Hermetic sealing



Tri-layered aluminium foil pouch

g) Long term storage (Base Collection)

The *ex situ* seed genebank at NBPGR comprises 12 long-terms modules (total capacity: 1 million accessions) maintained at -18^0C for hosing the base collections. Germplasm under long-term conservation is said to be a reserve against genetic losses and designated as base collection, the base collection is generally not distributed except for regeneration. Most of the seeds used in agriculture show orthodox behavior i.e they can withstand dehydration without loss of viability and such seeds can be stored in dry state on long-term bases. Longevity of seeds can be prolonged by decreasing the moisture content and storage temperature (sub zero temperature)

h) Documentation

Data documentation is the most important and dynamic activity. Correct and reliable recording of data, its documentation and information transfer is as important as proper handling of germplasm itself. Proper documentation of plant genetic resources allows the best use of data, it's easy retrieval and usefulness. This way the documentation of plant genetic resources is very useful in disseminating information on germplasm holdings more effectively and for a specific purpose enabling one to a search information having desired traits so that the selected accessions could be used for breeding programmes for developing new traits. It also acts as a source of information to assist in planning and operation of any gene bank activity. For documentation of gene bank holdings in NGB, two types of information files are used: passport data descriptors and Gene bank management descriptors.

The passport data descriptors include Name of Crop, Taxonomic Code, Cultivar Name, National_ID Number, Collector_No, Other_ID, Location in the Gene bank, while Gene Bank management descriptors include seed quantity, seed germination%, seed moisture % d source of material and date of storage etc. Being the most important component of National Genebank, it is accomplished efficiently through a well managed computerized network.

Reference

International Rules for Seed Testing (ISTA) 2015, International Seed Testing Association, Basserdorf, Switzerland.

International Plant Genetic Resources Institute (IPGRI) 1994, Genebank Standards, Food and Agriculture Organization of the United Nations, Rome.

Fig 1- Processing of seed material in NGB