3. Cleaning

The cost of maintaining accessions in genebanks is high. Only clean and high-quality seeds should be maintained in storage.

3. SEED CLEANING

What is seed cleaning?

Seed cleaning is the removal of debris, inert material, damaged and infected seeds, and seeds of other species to improve the quality of samples for storage (see Flowchart 3.1).

Why clean seeds?

Seed cleaning is necessary to:

- reduce bulk during transportation by removing extraneous materials;
- improve sample purity by removing damaged and immature seeds; and
- optimize storage space and reduce costs.

In fruit crops, some pre-cleaning may be necessary to remove leaves and twigs in order to reduce bulk and prevent the possible spread of diseases and pests.

When to clean seeds

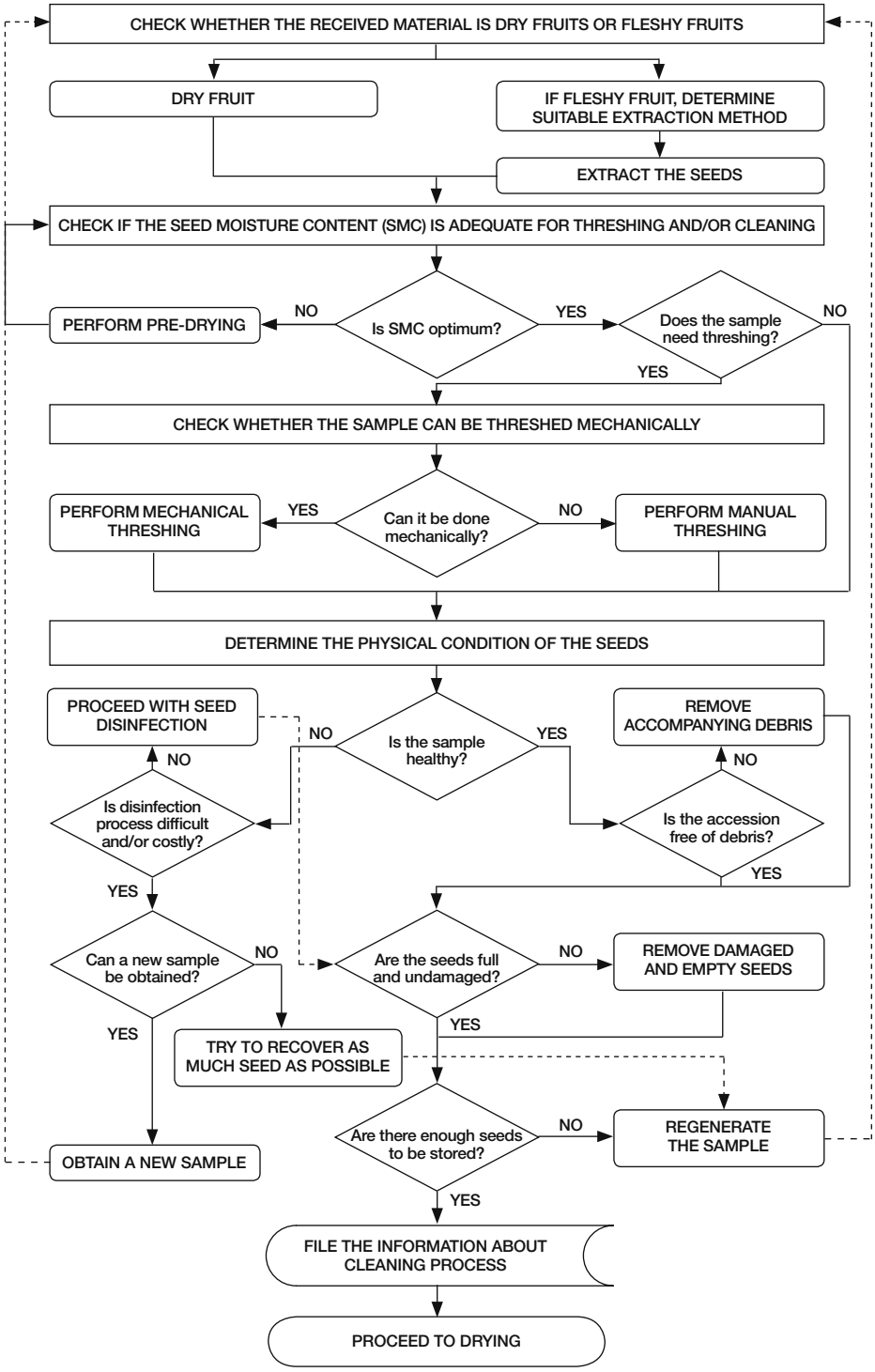
Seeds should be cleaned immediately after harvest or soon after they arrive at the genebank. Fruits may be soft and fleshy like drupes with fleshy pulp or hard and leathery like pods. Seed extraction is therefore the first step in seed cleaning.

If seeds cannot be handled immediately, fruits can be stored for a short time before seed extraction. Soft fruits are best stored at 10°–15°C in sufficiently high humidity to prevent drying. Hard or dry fruits are best stored in the shade in thin layers. It is essential that air circulates freely between moist fruits. To facilitate this, fruits should be held in ventilated containers such as trays with holes or wire-mesh bottoms, or in nylon-net bags.

Extracting seeds from fruits

Seeds should be mature before extraction. If not, it may be possible to ripen the fruits with the seeds inside by leaving them in a cool, well-ventilated environment. Storage conditions should simulate those on the parent plant. Seed-extraction procedures vary according to type of fruit.

Flowchart 3.1. Seed cleaning.



Extraction of seeds from dry dehiscent fruits

Genebanks generally receive seeds from dry dehiscent fruits after threshing. In some cases however, they are received in fruits as seed heads or as inflorescences, requiring separation from the vegetation.

Many dry dehiscent fruits (capsules, silique, follicles and dehiscent pods) open readily during drying when spread out in thin layer with sufficient air circulation. The physical release of seeds from fruits varies with species. In some, a minor movement such as raking, shaking or tumbling is sufficient for complete extraction. Seeds of some species such as some legumes maintain a strong attachment through the funicle and seeds may require extraction by hand or by threshing. Threshing is also required when seeds are received as heads (maize, pearl millet, etc.); it should be done when seed moisture content is between 12% and 16% in order to minimize injury to the seeds.

Seeds can be threshed either by hand or mechanically.

- Hand threshing is the preferred method because there is a lower probability of damage to seeds during the threshing process. Seeds can be threshed by placing them in sacks or spreading them on a threshing floor and beating them with sticks. Another method to remove seeds with strong attachments to pods is to rub them gently between two rough surfaces, such as rubber, sandpaper or stones, taking care not to scarify or crush the seeds.
- When using mechanical threshers, it is essential that the threshing machines are cleaned with a brush or air blower between lots to:
  - avoid contamination with seeds of accessions previously threshed; and
  - prevent diseases and pests from being passed from one accession to another.

Extraction of seeds from dry indehiscent fruits

Some indehiscent fruits may need to be broken mechanically in order to extract their seeds. Some initial drying is necessary to promote brittleness and facilitate subsequent extraction.

- Seeds from larger fruits (such as groundnut and beans) can be extracted by splitting each fruit by hand or by mechanical treatment without damaging the seeds.
- Smaller indehiscent fruits (such as chickpea and brassicas) may be broken up by threshing as described above.
- Pods with gummy material (such as *Prosopis cineraria*) require several rounds of threshing and intermittent drying.

Sweating technique for forage grasses

Sweating is a useful technique for improving the maturity and easing the threshing and cleaning of some tropical grass seeds that are closely held in glumes. It consists of stacking the freshly cut heads, wrapping them in grass or a tarpaulin to allow heating or sweating under shade, and preventing them from drying for three to four days. After this time, the mature seeds are easily shed without threshing. The stack must be watched closely and turned occasionally to prevent overheating; excessive temperatures during sweating may cause seed deterioration.

Extraction of seeds from fleshy fruits

The method of extraction from fleshy fruits varies with the type of fruit.

- Seeds are best removed by cutting the fruit in half or by cutting off the distal end and squeezing out the contents into a container.
- Small seeds of pulpy fruit can be extracted by mashing the pulp, mixing it with water, allowing the seeds to settle and then pouring off the pulp.
- Large seeds can be teased out from pulp with forceps (such as *Citrus* spp.). Pulp can also be detached by washing the seeds in sieves under running water or by rubbing them against wire mesh and rinsing to remove the pulp. A blender can be used to mash large quantities of pulp, but it is easy to over-blend and damage the seeds. Use brief, intermittent agitation at low speeds. Covering blender blades with rubber coating also minimizes damage. Hand-processing is preferable to avoid physical damage to seeds during this process.
- After washing, dry the seeds in thin layers on absorbent sheets with circulating air in the shade, avoiding heat.

Mucilaginous seeds

If mucilage surrounds the seeds (such as tomato, cucumber and some melons) and cannot be removed by washing, a number of options exist:

- Gently rub the wet seeds on a wire-mesh screen (the mesh size should retain seeds while the pulp passes through) with a gloved hand.
- Gently rub the seeds with clean, coarse sand, then wash off the sand and mucilage.
- It is also possible to dry the seeds first and then rub the dry mucilage off. Ensure that the seeds do not stick to the drying surface and that they are well separated to prevent sticking together during drying.
- To remove mucilage, fermentation of the gelatinous slurry (at 20°–25°C for up to three days), acid treatment (2–4% hydrochloric acid solution added to the slurry in the ratio of 1:1 for one hour), enzymatic digestion (pectinase solution, 0.1% weight/volume added to the slurry in the ratio of 1:40 for 24 hrs) and sodium

bicarbonate (10% solution mixed with the slurry in the ratio of 1:1 and left for 18–24 hours) are also used. Prolonged treatments can damage seeds, however, and should be used with caution.

Fruits with pulp firmly attached

Fruits in which pulp is firmly attached to seeds (such as almond) can be processed by the following methods:

- Soak the fruit in buckets or other suitable containers until they are soft, but not so long that they start to ferment, as indicated by bubbles and odour. Separate the seeds from the pulp by hand.
- Mash the soaked fruits to separate the flesh from the seeds.

After de-pulping, wash the seeds thoroughly to clean away all traces of pulp. Washing under a stream of water is the best method.

Stone fruits

Stone fruits (such as peach, plum and apricot) can be de-pulped in a food processor (blades should be protected by rubber) without risk of damaging the seeds. Removal of flesh with a sharp knife by hand is also convenient for small quantities of seeds. After de-pulping, wash the stones in running water to remove traces of pulp and blot the surface dry. If seeds have to be extracted for storage, dry the stones and split each endocarp with pliers, applying pressure at the broadest point of the longitudinal axis of the stone. Alternatively, insert a strong blade into the crevice and twist.

Do not attempt to dry seeds if they are known to be recalcitrant and cannot survive desiccation to low moisture content.

It is important that orthodox seeds removed from fruits are dried quickly at appropriate temperatures to low moisture contents for long-term storage.

How to clean seeds

Cleaning should not cause damage to samples or lead to waste. It can be done manually or by machines, but genebanks are strongly advised to clean accessions by hand for the following reasons:

- Mechanical cleaning could result in selection within genetically heterogeneous accessions (due to exclusion of very small and very large seeds passing through mechanical apertures).
- Equipment requires rigorous cleaning and often careful adjustment between accessions.

Step 1: Separation from debris

The first step in seed cleaning is to remove all debris (non-seed material) from the entire sample.

- Use hand sieves with different graded mesh sizes to remove large and fine debris. When cleaning genetically heterogeneous accessions, it is important to return small seeds to accession bulk.

- Separate empty seeds and other light material like chaff that has not been separated in the sieving process by gentle winnowing[2] or in a seed blower.[3]

Step 2: Examining seeds for insect and fungal damage

- Spread the seeds on a flat, well-lit surface of contrasting colour and observe any visible signs of infestation. Use an illuminated table or purity workboard if available.
- If seeds are found to be mouldy or infested:
  - isolate the affected sample from rest of the material;
  - dry the seeds to low moisture content in sealed containers with silica gel to prevent further spread of fungi or insects;
  - if infestation is suspected, store the seeds at sub-zero in a freezer for seven days to kill insects before removing infected seeds and continuing with normal packaging and storage procedures.

Step 3: Examining seeds for mechanical damage and empty seeds

- Spread the seeds on a flat well-lit surface of contrasting colour, such as an illuminated table or purity workboard.
- Examine for physical damage or any empty seeds.
- Manually separate and discard any visually damaged or shrivelled seeds.
- Separate empty seeds and light material by blowing as described above.

Step 4: Purity analysis

Purity is an expression of how 'clean' the seed lot is. Information on actual seed lot composition is important; purity analysis serves as a guideline to determine the necessity of further cleaning. During purity analysis, each 'pure' seed fraction[4] from the working sample is separated from the inert matter and other seeds.

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[2] Seeds are held in flat baskets and thrown up into the air. The wind blows away light matter like dust and leaf fragments while the heavier seeds fall back into the basket.

[3] Seed lots are placed in the vertical cylinder connected to an electric-powered air current at the bottom. The upward air current displaces all light material like chaff to the top while heavier seeds are collected at the bottom.

[4] ISTA (2005) specifies a pure seed faction to contain: (i) intact seeds of actual species as well as dead, shrivelled, diseased, immature and pre-germinated seeds; (ii) achenes and similar fruits. such as samara with or without perianth regardless of whether they contain a true seed, unless it is apparent that none is contained; and (iii) fractions of broken seeds, achenes, etc. that are more than half of the original size. In genebanks, purity should be attributed to samples that are not only free from seeds of weeds and other crop species, debris and inert material, but also from empty, immature, damaged and infected seeds.

Purity should be attributed to samples that are not only free from seeds of weeds and other crop species, debris and inert material, but from empty, immature, damaged and infected seeds. Genebanks should aim for absolute purity – it is important to set standards as high as 95% for the proportion of pure seeds in accessions. If an accession fails to meet this target after the initial cleaning, it should then be re-cleaned as many times as necessary for absolute purity.

- Weigh out a working sample of given weight (for example 250 g) of the total seed lot randomly using an electronic balance.
- Spread the sample on table and separate out all pure seeds manually with tweezers or remove impurities by blowing, sifting or letting seeds roll down a slanting surface.
- Weigh the 'pure' seed fraction and express purity as the percentage weight of pure seed over the total weight of the working sample, as shown below.

$$\text{Purity (\%)} = \frac{\text{Weight of pure seeds (g)}}{\text{Total weight of working sample (g)}} \times 100$$

Example:

Total weight of working sample = 250 g
Weight of pure seeds = 245.2 g
Inert matter = 3.5 g
Other seeds = 1.3 g

$$\text{Purity (\%)} = \frac{245.2 \times 100}{250} = 98.08\%$$

Step 5: Verification

After cleaning:

- Check the samples again visually for purity and damaged seeds.
- Check the reference sample (see Chapter 6) or reference data for matching seed colour and shape if the samples are received after regeneration.
- Carefully destroy the waste material to prevent the spread of insects and diseases to other material.

Useful equipment

The following equipment is useful for seed cleaning:

- Sieves: A set of stacking graded sieves such as those used for soil-testing. The most useful sizes are standard numbers 5, 10, 18, 35, and 60, corresponding to hole sizes of 0.1574"/4 mm, 0.787"/2 mm, 0.394"/1 mm, 0.197"/0.5 mm, and 0.0098"/0.25 mm. Kitchen sieves with different grate sizes or a coarse-weave cloth can also be used.
- Glass measuring cups in a range of sizes: Pyrex 1-, 2-, and 4-cup measuring cups
- Small trays, mixing bowls, strainers, colanders and other plastic containers; ordinary kitchenware works well
- Cutting tools: a sharp knife, a serrated-edge knife, razor blades or a razor knife with disposable blades, fine-tipped pruning shears

- Cutting board
- Vice-grip pliers
- Files, sandpaper, wire gratings, and other abrading tools
- Filter funnels such as Melitta number 6 coffee funnels and filter paper
- Magnifier lamp, headband magnifier, and 7–14x hand lens
- Forceps, tweezers, needle-nosed pliers
- Hand hair blow-drier, preferably multi-speed with heater unit disabled
- Seed blower—a mechanical device for winnowing seed to reduce the amount of waste, especially in grass seed (e.g. South Dakota blower)
- Small fan
- Blender with rubber-coated blades
- Spray bottles with adjustable nozzles

Documentation

Many genebanks tend not to document the seed-cleaning procedures except for the date of seed cleaning. As germplasm collections often encompass a variety of fruit and seed characteristics, and cleaning procedures vary according to crop and accessions, it is important that all associated data are captured and stored for future reference. The following descriptors may be used to document accession-level information on seed cleaning:

- Type of sample
- Method of extraction
- Method of threshing
- Method of cleaning
- Date of cleaning
- Proportion of empty, immature or damaged seed (%)
- Total seed number or weight after cleaning
- Seed purity (%)

Further reading

Ellis, R.H., Hong, T.D. and Roberts, E.H. 1985. Handbook of Seed Technology for Genebanks. Volume 1. Principles and methodology. IBPGR, Rome, Italy.

ISTA. 2005. International Rules for Seed Testing. Edition 2005. International Seed Testing Association, Bassersdorf, Switzerland.

Schmidt, L. 2000. Guide to handling of tropical and subtropical forest seed. Danida Forest Seed Centre, Humlebaek, Denmark.

In genebanks, moisture content is usually expressed on a wet-weight basis

4. SEED MOISTURE CONTENT DETERMINATION AND DRYING

4.1 Seed moisture content determination

What is seed moisture content?

Seed moisture content (SMC) is the amount of water in a seed. Water is present both in free form and bound to chemical compounds in cells such as carbohydrates and protein.

SMC is expressed in terms of the weight of water contained in a seed as a percentage of the total weight of the seed before drying, known as the wet-weight (wb) or fresh-weight basis (International Seed-Testing Association [ISTA] 2005).

$$\text{SMC (\% wb)} = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100$$

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

$$\text{SMC (\% db)} = \frac{\text{wet weight} - \text{dry weight}}{\text{dry weight}} \times 100$$

Why is it important to determine seed moisture content?

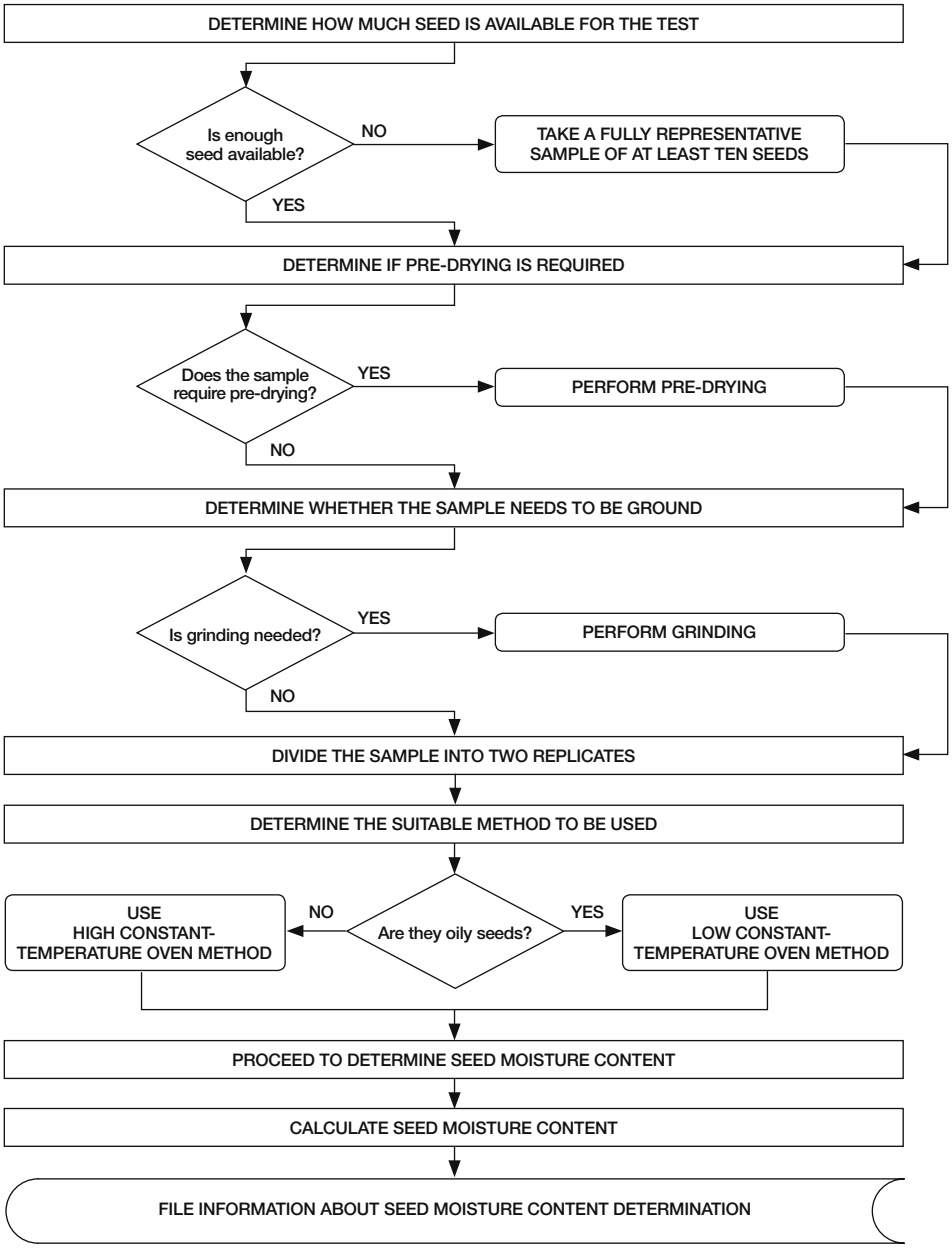
Moisture content is the most important factor determining the rate at which seeds deteriorate and has profound impacts on storage longevity of seeds in genebanks. Even small changes in moisture content have large effects on storage life. It is important to determine moisture content before storage in order to accurately predict the potential storage life of each accession.

Determining seed moisture content

Seed moisture content can be determined by two different methods (see Flowchart 4.1):

- the oven-drying method, described by ISTA (2005); and
- moisture meters.

Flowchart 4.1. Seed moisture content determination.



Oven-drying method

The most accurate method for determining moisture content is the oven-drying method, in which water is removed from seeds by heat under controlled conditions. This method is destructive to seeds and should be carried out only when essential. It is recommended that one accurate determination be conducted using this method after the drying period to determine the initial moisture content of the stored seeds.

ISTA (2005) 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.

The recommended method for drying important crop and forage species is given in Table 4.1.

Table 4.1. Suggested method of moisture determination for important crop and forage species (ISTA, 2005).

Low constant temperature oven method		
Brassicas	Falseflax (*Camelina*)	Sesame (*Sesamum*)
Castor (*Ricinus*)*	Flax (*Linum*)	Soya bean (*Glycine*)*
Pepper (*Capsicum*)	Groundnut (*Arachis*)*	All tree species
Cotton (*Gossypium*)*	Onion (*Allium*)	
Eggplant (*Solanum*)	Radish (*Raphanus*)	
High constant temperature oven method		
Alfalfa (*Medicago*)	Cocksfoot (*Dactylis*)	Rye (*Secale*)*
Asparagus (*Asparagus*)	Cress (*Lepidium*)	Ryegrass (*Lolium*)
Barley (*Hordeum*)*	Crested dogtail (*Cynosurus*)	Sainfoin (*Onobrychis*)
Bean (*Phaseolus*)*	Cucumber (*Cucumis*)	Serradella (*Ornithopus*)
Beet (*Beta*)	Cumin (*Cuminum*)	Sorghum (*Sorghum*)*
Bentgrass (*Agrostis*)	Dallisgrass (*Paspalum*)	Squash (*Cucurbita*)
Bermuda grass (*Cynodon*)	Fescue (*Festuca*)	Sweetclover (*Melilotus*)
Black salsify (*Scorzonera*)	Foxtail (*Alopecurus*)	Tall oatgrass (*Arrhenatherum*)
Bluegrass (*Poa*)	Lettuce (*Lactuca*)	Timothy grass (*Phleum*)
Brome (*Bromus*)	Lupin (*Lupinus*)*	Tomato (*Lycopersicon*)
Buckwheat (*Fagopyrum*)*	Maize (*Zea*)*	Trefoil (*Lotus*)
Canarygrass (*Phalaris*)	Millet (*Panicum*)	Tufted hairgrass (*Deschampsia*)
Caraway (*Carum*)	Oat (*Avena*)*	Velvetgrass (*Holcus*)
Carrot (*Daucus*)	Parsley (*Petroselinum*)	Vetch (*Vicia*)*
Chervil (*Anthriscus*)	Pea (*Pisum*)*	Watermelon (*Citrullus*)*
Chicory (*Cichorium*)	Rhodes grass (*Chloris*)	Wheat (*Triticum*)*
Chickpea (*Cicer*)*	Rice (*Oryza*)*	
Clover (*Trifolium*)		

*grinding required.

Pre-drying

Pre-drying is obligatory if seeds are wet and their moisture content is suspected to be above 17% (10% for soya bean and 13% for rice); it should be conducted prior to moisture content determination by oven-drying. If pre-drying is required, proceed as follows:

1. Weigh two sub-samples of 4–5 g of seeds in their containers.
2. Pre-dry the samples overnight in a warm, dry place such as a laboratory bench.
3. Weigh them again in their containers and determine the loss of weight (loss of moisture) by subtraction.
4. Calculate the moisture content on a fresh-weight basis.

Equipment

The following equipment is necessary for determining moisture content by oven-drying:

- a mechanical-convection (forced-draught) oven with a recovery time of 15 minutes or less, capable of maintaining the required temperature within 1°C and fitted with a thermometer accurate to 0.5°C;
- non-corrosive drying containers (metal or glass) with tight fitting lids—the size of the container should allow the height of the evenly-distributed sample to be under 0.3 g cm^{-2};
- a grinder with adjustable speeds to obtain specified particle sizes (0.5–4.0 mm)—it should not cause undue heating while grinding;
- an analytical balance that is capable of weighing to 3–4 decimal places (0.001–0.0001 g);
- a desiccator fitted internally with a thick metal or ceramic plate to promote rapid cooling of the containers, and containing a desiccant such as silica gel or calcium chloride at the bottom; and
- tongs or gloves to handle hot containers.

Sample size and sampling

The oven-drying method is destructive and considering that seed quantity is limited in most genebanks, small sample weights should be used.

1. Use two independent replicates of 0.5–1.0 g of seeds or a minimum of ten seeds for moisture determination depending on availability.
2. The sample should be representative of the entire accession. Make sure that the seed lot is well mixed and that the sample is drawn from small portions in different positions of the seed lot.
3. Once sampled, keep the seeds in moisture-proof containers until they are tested to avoid changes in moisture content.

Remember that if the seed lot comes from cold storage, water may condense on the seeds. When sampling, do not open the containers until they have reached room temperature.

Grinding

Some seeds require grinding into smaller particles to promote uniform and complete drying. A list of species that require grinding is given in Table 4.2.

Table 4.2. Species for which grinding is obligatory (ISTA, 2005).

Arachis hypogaea	*Gossypium* spp.	*Pisum sativum*
Avena spp.	*Hordeum vulgare*	*Secale cereale*
Cicer arietinum	*Lathyrus* spp.	*Sorghum* spp.
Citrullus lanatus	*Lupinus* spp.	*Triticum* spp.
Fagopyrum esculentum	*Oryza sativa*	*Vicia* spp.
Glycine max	*Phaseolus* spp.	*Zea mays*

Moisture content determination

High constant temperature method for non-oily seeds

Moisture content is determined in the following way:

1. Dry the containers at 130ºC for one hour and allow them to cool in the desiccator for one hour.
2. Label and weigh each container, including the lid, and record the weights on the data sheet shown in Table 4.3 (column W1). For accuracy in moisture determination, the size and weight of the containers should be relative to the sample weight used.
3. Place two 0.5–1.0 g sub-samples, randomly selected from each sample (pre-dried and ground if necessary), into two separate containers, which will serve as two replicates. Replace the lids, weigh again and record the weights in Table 4.3 (column W2).
4. Place the containers with the lids removed in an oven maintained at 130°–133ºC.
5. Dry the seeds for one to four hours depending on the species (four hours for *Zea mays*, two hours for other cereals and one hour for other species).
6. Replace the lid on each container at the end of the drying period.
7. Move the containers to a desiccator and allow them to cool for 45 minutes.
8. Record the weight of the containers, including the samples, in Table 4.3 (column W3).
9. Calculate the moisture content on a wet-weight basis and express it as a percentage to one decimal place, using the following formula:

   $$\text{Moisture content (\%)} = \frac{W2 - W3}{W2 - W1} \times 100$$

   where,

   W1 = weight of container with lid;
   W2 = weight of container with lid and sample before drying; and
   W3 = weight of container with lid and sample after drying.
10. Repeat the test if the moisture content between the two replicates differs by more than 0.2%.

The drying period commences when the oven has attained the required temperature after the samples are kept in the oven and the oven door is closed.

Table 4.3. Recording and calculation of seed moisture content.

Accession no.	Replicate/ container no.	Wt of empty container with lid (g)	Wt of container with lid + seed before drying (g)	Wt of container with lid + seed after drying (g)	Moisture content % (wb)	
		W1	W2	W3	(W2-W3)/ (W2-W1) × 100	Average (R I + R II)/2
	R I					
	R II					
	R I					
	R II					
	R I					
	R II					
	R I					
	R II					

Example:

Accession no.	Replicate/ container no.	Wt of empty container with lid (g) (W1)	Wt of container with lid + seed before drying (g) (W2)	Wt of container with lid + seed after drying (g) (W3)
	R 1	10.3245	14.8668	14.4356
	R 2	10.1442	14.9948	14.5365

Calculation:

Rep 1:

$$\% \text{ Moisture content} = \frac{14.8668-14.4356}{14.8668-10.3245} \times 100 = 9.47$$

Rep 2:

$$\% \text{ Moisture content} = \frac{14.9948-14.5365}{14.9948-10.1442} \times 100 = 9.45$$

$$\text{Moisture content (fresh-weight basis)} = \frac{9.47+9.45}{2} = 9.46\%$$

If samples have been pre-dried, use the following formula to determine the final moisture content.

Final moisture content (%) = $(M1+ M2) - \frac{(M1 \times M2)}{100}$ where,

M1 = percentage moisture content from first-stage drying (pre-drying)
M2 = percentage moisture content from second-stage drying (oven drying)

During moisture determination, exposure of the sample to the laboratory environment should be reduced to a minimum.

Low constant temperature method for oily seeds

For oily seeds, use a lower temperature for a longer period so only water is lost from the seeds. Follow the procedure described above, except for steps 4 and 5, which should be modified as follows:

1. Place the container with the lids removed in an oven maintained at 103°±2ºC.
2. Dry seeds for 17±1 hours.

Use of a higher temperature and longer drying period than normally recommended will lead to loss of volatile compounds and water, particularly in oil-rich seeds. This will result in an over-estimation of the moisture content.

Moisture balances

Moisture balances combine state-of-the-art heating with highly accurate weighing for a fast and precise method of moisture analysis. Using the principle of loss of weight upon drying—the standard for moisture measurement—the balance automatically weighs a sample, dries it, measures the weight loss due to drying and calculates the moisture content of the seeds. The analysis will automatically terminate when drying is complete and the dry weight is stable, or after an amount of time specified by the operator. The final result is shown on the digital display.

The major disadvantage of the oven-drying and moisture-balance methods, particularly when dealing with accessions containing limited numbers of seeds, is that seeds are killed at temperatures used for drying; these methods are also time-consuming. Several genebanks use rapid and non-destructive methods to circumvent these problems, although some of these methods are less accurate than the oven-drying method.

Non-destructive methods for moisture determination

Quick moisture meters

Seed moisture content can also be determined by using quick moisture meters. A variety of quick moisture meters are available. They measure the electric properties of seed moisture either by conductivity[5] or capacitance.[6] It is important to note that these meters need to be calibrated using the standard oven-drying method for each crop tested, and are more accurate for moisture contents in a specified range (6–25%) depending on the type of moisture meter used. They are less reliable above and below this

[5] Conductivity is a measure of the electrical resistance of seed material.

[6] Capacitance is a measure of the ability of seeds to store electrical charge.

range. It is recommended to use moisture meters only for a rough determination of moisture content before drying.

Calibration of quick moisture meters

The exact relation of a moisture meter reading to actual seed moisture, as determined by the ISTA oven method, is called calibration. Calibration should be based on many samples from different varieties, areas and years, and should include the range of moisture contents normally encountered for the species (6–25%). Calibration curves are established by plotting readings from the moisture meter against those obtained by the oven-drying method. Once a calibration curve is established, any reading can be easily converted to actual moisture content.

To determine moisture content using a calibrated moisture meter, proceed as follows:

1. Take two randomly selected samples from the seed lot that have the weight and volume required for the specified meter.
2. Place the sample in the seed chamber and record the reading.
3. The moisture content (as a percentage by weight) is equal to the mean of the readings of the two samples tested.

Digital humidity sensors

Many genebanks now use digital humidity sensors for moisture content determination. These methods rely on the fact that seeds gain or lose moisture rapidly depending on their surroundings. Moist seeds in dry air lose moisture; dry seeds in moist air gain moisture. After a sufficient amount of time, there is no further movement of moisture between seeds and air; at this point, seeds are said to be at equilibrium.

Digital humidity sensors measure the amount of water vapour in the air at equilibrium with a sample of seeds enclosed in a sealed chamber. The reading is generally expressed as equilibrium relative humidity (eRH), and may be related to conventional moisture content using a calibration curve developed using the procedure above.

Further reading

Ellis, R.H., Hong, T.D. and Roberts, E.H. 1985. Handbook of Seed Technology for Genebanks. Volume 1. Principles and methodology. IBPGR, Rome, Italy.

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Probert, R.J., Manger, K.R. and Adams, J. 2003. Non-destructive measurement of seed moisture. Pp. 367-387 in Seed conservation: Turning science into practice. (R.D. Smith, J.B. Dickie, S.H. Linington, H.W. Pritchard and R.J. Probert, eds.). Royal Botanic Gardens, Kew, UK.

4.2 Seed drying

What is seed drying?

Seed-drying is the reduction of seed moisture content to recommended levels for storage using techniques that are not detrimental to seed viability (see Flowchart 4.2).

Why are seeds dried?

Freshly harvested seeds can have high moisture contents, which promote respiration and growth of seed embryos, insects and fungi. Seeds must therefore be dried to a safe moisture content to prevent damage, heating and infestation during storage.

When are seeds dried?

The drying of a seed sample should start as soon as possible after receipt of the seeds to avoid deterioration. It is important to ensure that seeds are not left in sheds, stores or corridors, but placed in a well-aerated and cool environment (with low relative humidity) immediately upon arrival at the genebank. In a room with a high relative humidity, a mechanical device to remove moisture (dehumidifier) may be required.

To what moisture content should seeds be dried?

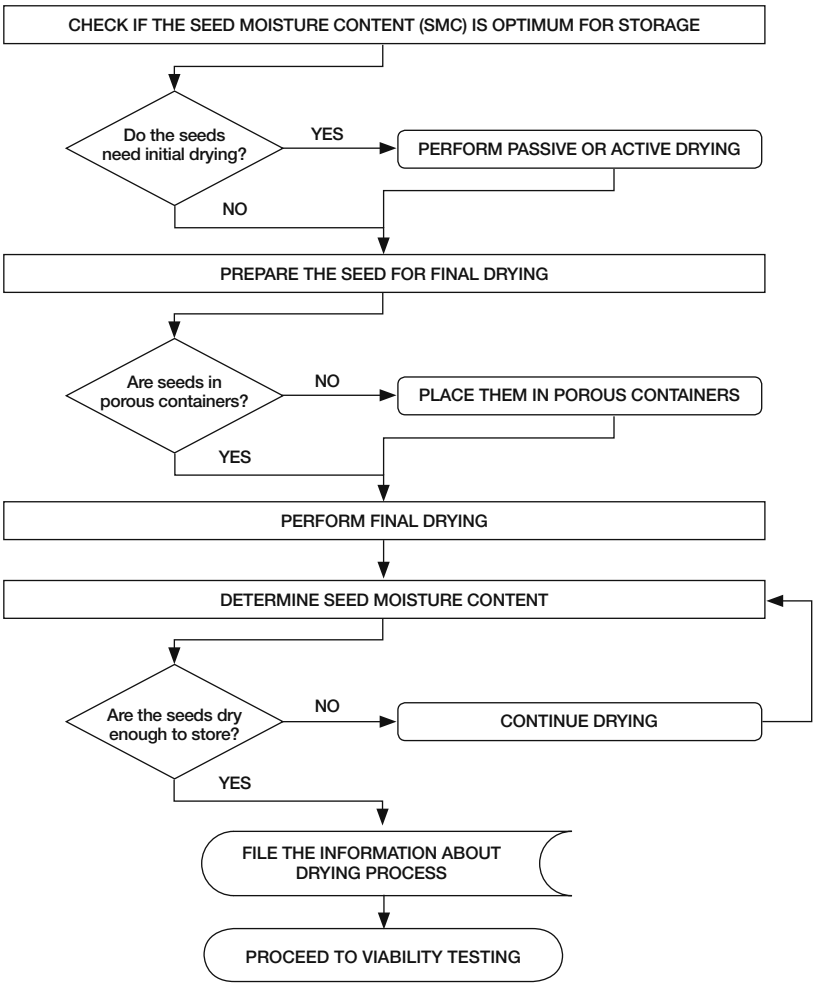
The optimal moisture content for storage depends on the species and the intended period of storage. It is important to adopt an appropriate drying regime, in which the relative humidity and temperature of the drying air are regulated to achieve the target moisture content.

- The moisture content of seeds to be stored as base collections (see glossary) should be between 3% and 7%, depending on the species.[7]
- The moisture content of seeds to be stored as active collections (see glossary) should be between 3% and 8% for seeds with poor storage characteristics (such as oily seeds) and between 7% and 11% for seeds with good storage characteristics (such as cereals), depending on the temperature used for storage. (For further information, see Table 6.2 in Chapter 6.)

[7] For base collections, equilibrium seed moisture contents at 10-15% relative humidity are recommended for seed drying (see *Dehumidified drying* in this section). Equilibrium seed moisture content depends on lipid content – seeds with a high oil content will have a lower moisture content than will starchy seeds at the same relative humidity since the oil volume in the seed excludes water (see table 4.4). If seed oil content (D_0) is known, Cromarty et al. (1992) provide an equation to estimate the equilibrium seed moisture content (M_e, dry weight basis) at a given relative humidity (R as a decimal) and at a given temperature (T in °C).

$$M_e = \frac{(1-D_0) \times \sqrt{(-440 \times \ln(1-R))}}{1.1+(T/90)}$$

Flowchart 4.2. Seed drying.



Critical moisture content

The critical moisture content is the level below which further reduction in moisture content no longer increases seed longevity in hermetic storage. Ellis, Hong and Roberts, working since 1988 with more than 25 crop species, found that hermetic storage at critical moisture content provides maximal seed longevity at a given storage temperature. Critical moisture content values vary with species, from about 6% for pea (*Pisum sativum*) and mungbean (*Vigna radiata*), which are rich in protein, to 4.5–5.0 for cereals like rice, wheat and barley, which are rich in starch. For oily-seed species, the values of critical moisture content are lower: 3.3% for soya bean (*Glycine*

max); 2.7% for flax (*Linum usitatissimum*); 2.4% for niger (*Guizotia abyssinica*); and 2% for groundnut (*Arachis hypogaea*) and sunflower (*Helianthus annuus*). These values were determined by storing seeds at 65°C, after equilibration with 10–11% relative humidity (RH) at 20°C However it has been reported that critical moisture contents are affected by temperature and caution must be exercised when extrapolating data from accelerated ageing studies to actual seed storage conditions because the thermodynamic conditions of the two environments may be quite different (see Vertucci and Roos, 1993). For more specific information on critical moisture content for different species, see Ellis (1998), Ellis et al. (1989, 1990 and 1996) and Walters (1998 and 2003). Physical damage and cracking of seed coats can be caused by rapid or overdrying of seeds in a few species like soya bean, groundnut, chickpea and *Sterculia foetida.* To avoid this form of damage, seeds of sensitive species should be dried carefully in a stepwise manner; with an initial slow drying at slightly higher relative humidity, followed by a second-stage drying.

Principles of seed drying

Seeds are hygroscopic and absorb or give off moisture depending on the relative humidity of the surrounding air and the gradient in water potential between the seed and surrounding air. If the water vapour pressure of the seed is greater than the surrounding air, the seed will lose moisture and become drier (desorption). If the water-vapour pressure of a seed is lower than that of the surrounding air, the seed will gain moisture by absorption. Absorption or desorption occurs until the water vapour pressure in the seed and the surrounding air are balanced.

Equilibrium moisture content and moisture isotherms

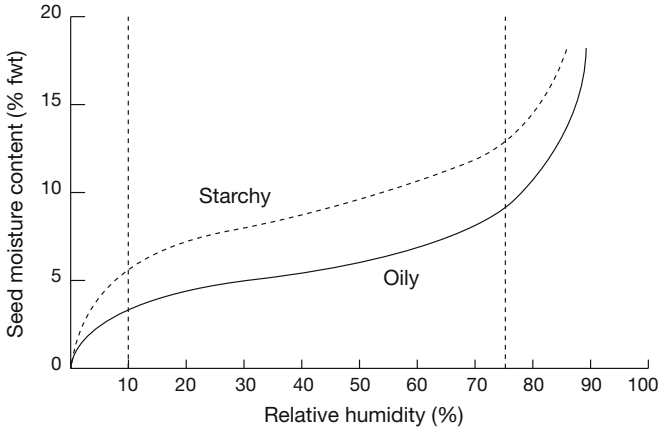
The water content of seeds at equilibrium with the relative humidity of the surrounding air is referred to as equilibrium moisture content. Understanding the relationship between equilibrium seed moisture content and relative humidity is important in determining the appropriate drying regime for seeds.

For a given species, there is a definable relationship between relative humidity and seed moisture content (see Table 4.4). Seeds will lose or absorb water until their moisture content is in balance with the RH of the surrounding air at that temperature. The relationship between seed moisture content and relative humidity is expressed by a sorption isotherm—this is simply a graph of seed moisture content against percentage relative humidity (see Figure 4.1). Moisture isotherms depend on the chemical composition of seeds and differ between species, between accessions of the same species and even between seeds of the same accession harvested

Table 4.4. Equilibrium moisture contents (approximate) of some common crop seeds at 25°C.

Species	RH (%)							
	10	15	20	30	45	60	75	90
Barley	-	6.0		8.4	10.0	12.1	14.4	19.5
Bean, lima	4.6	-	6.6	7.7	9.2	11.0	13.8	-
Beet	2.1	-	4.0	5.8	7.6	9.4	11.2	
Buckwheat	-	6.7	-	9.1	10.8	12.7	15.0	19.1
Cabbage	2.9		4.6	5.4	6.4	7.6	9.6	-
Carrot	4.5	-	5.9	6.8	7.9	9.2	11.6	-
Cucumber	2.6	-	4.3	5.6	7.1	8.4	10.1	-
Egg plant	3.1	-	4.9	6.3	8.0	9.8	11.9	-
Flax	3.3	-	4.9	5.6	6.3	7.9	10.0	15.2
Groundnut	3.0	-	3.9	4.2	5.6	-	9.8	13.0
Lettuce	2.8	-	4.2	5.1	5.9	7.1	9.6	-
Maize	3.8	-	5.8	8.4	10.2	12.7	14.4	18.8
Mustard	1.8	-	3.2	4.6	6.3	7.8	9.4	-
Oat	-	5.7	-	8.0	9.6	11.8	13.8	18.5
Okra	3.8	-	7.2	8.3	10.0	11.2	13.1	-
Onion	4.6	-	6.8	8.0	9.5	11.2	13.4	-
Radish	2.6	-	3.8	5.1	6.8	8.3	10.2	-
Pea	5.4	-	7.3	8.6	10.1	11.9	15.0	-
Rice	4.6	5.6	6.5	7.9	9.8	11.8	14.0	17.6
Rye	-	7.0	-	8.7	10.5	12.2	14.8	20.6
Sorghum	-	6.4	-	8.6	10.5	12.0	15.2	18.8
Soya bean	4.1	-	5.5	6.5	7.4	9.3	13.1	18.8
Squash	3.0	-	4.3	5.6	7.4	9.0	10.8	-
Tomato	3.2	-	5.0	6.3	7.8	9.2	11.1	-
Turnip	2.6	-	4.0	5.1	6.3	7.4	9.0	-
Watermelon	3.0	-	4.8	6.1	7.6	8.8	9.0	-
Wheat	5.5	-	7.0	8.5	10.4	12.1	14.6	19.8

Compiled from: Roberts, E.H. (ed.). 1972. Seed Viability. Chapman and Hall, London; Harrington, J. F. 1972. Seed Biology, Vol III. Academic press, New York: 145–245; and Justice O.L. and Bass L.N. 1978. Principles and practices of seed storage, Agriculture Handbook No. 506. USDA, Washington D.C, USA.



Source: Bradford, K.J. 2004. Seed storage and longevity. pp 76–84. In: Seed production and quality. UC Davis, Seed Biotechnology Center, USA.

Figure 4.1. Moisture isotherms.

at different stages of development. Moisture isotherms are very useful in estimating the moisture content to which seeds can be dried in a given environment.

How to prepare moisture isotherms

Moisture isotherms can easily be constructed by allowing seeds to reach equilibrium in environments with known RH maintained by saturated salt solutions at a given temperature. The following saturated salt solutions provide a series of RHs at 25°C:

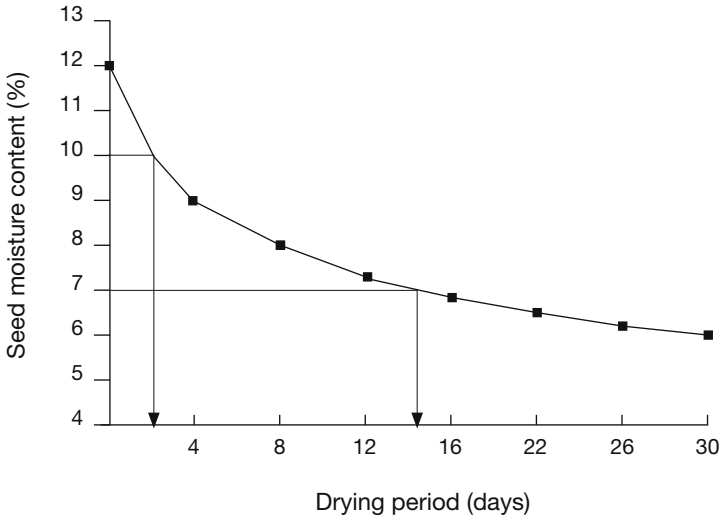
Salt	Corresponding RH (%)
Sodium hydroxide	7.5
Lithium chloride	13
Magnesium chloride	32
Magnesium nitrate	54
Ammonium nitrate	65
Sodium chloride	75
Potassium chloride	85

Saturated salt solutions are prepared by mixing salt with water to form a wet slurry.

1. Place the slurry at the bottom of a desiccator.
2. Place a known weight of seeds in wire-mesh containers or bags made of mosquito netting and keep them over the desiccator plate. The salt mixture should not come in contact with the seeds.
3. Seal the lid on the desiccator.
4. Allow enough time for seed moisture to equalize with the surrounding air inside the desiccator—this may take several weeks. Seeds will either absorb or lose moisture depending on the gradient in water pressure between the seeds and surrounding air. When the weight of seeds remains unchanged, equilibrium moisture content is attained.
5. Determine the equilibrium seed moisture content at each RH by oven-drying as described in the previous section. Plot the equilibrium seed moisture content on the Y-axis of a graph and the RH of the respective salt solutions on the X-axis as shown in Figure 4.2.

Assessing desiccation sensitivity

Testing seeds for desiccation tolerance is a prerequisite for choosing the appropriate drying regime if desiccation behaviour is not yet known. Recalcitrant seeds cannot survive desiccation below comparatively



Example: Seeds received at the genebank have an initial moisture content of about 10%, and need to be dried to 7% moisture content for storage. On the above graph, the lines from the curve to the time axis (X-axis) indicate two and 15 days, approximately. The difference between the two values (15 –2 = 13 days) is the time required to dry the seeds from 10% moisture content to 7% moisture content.

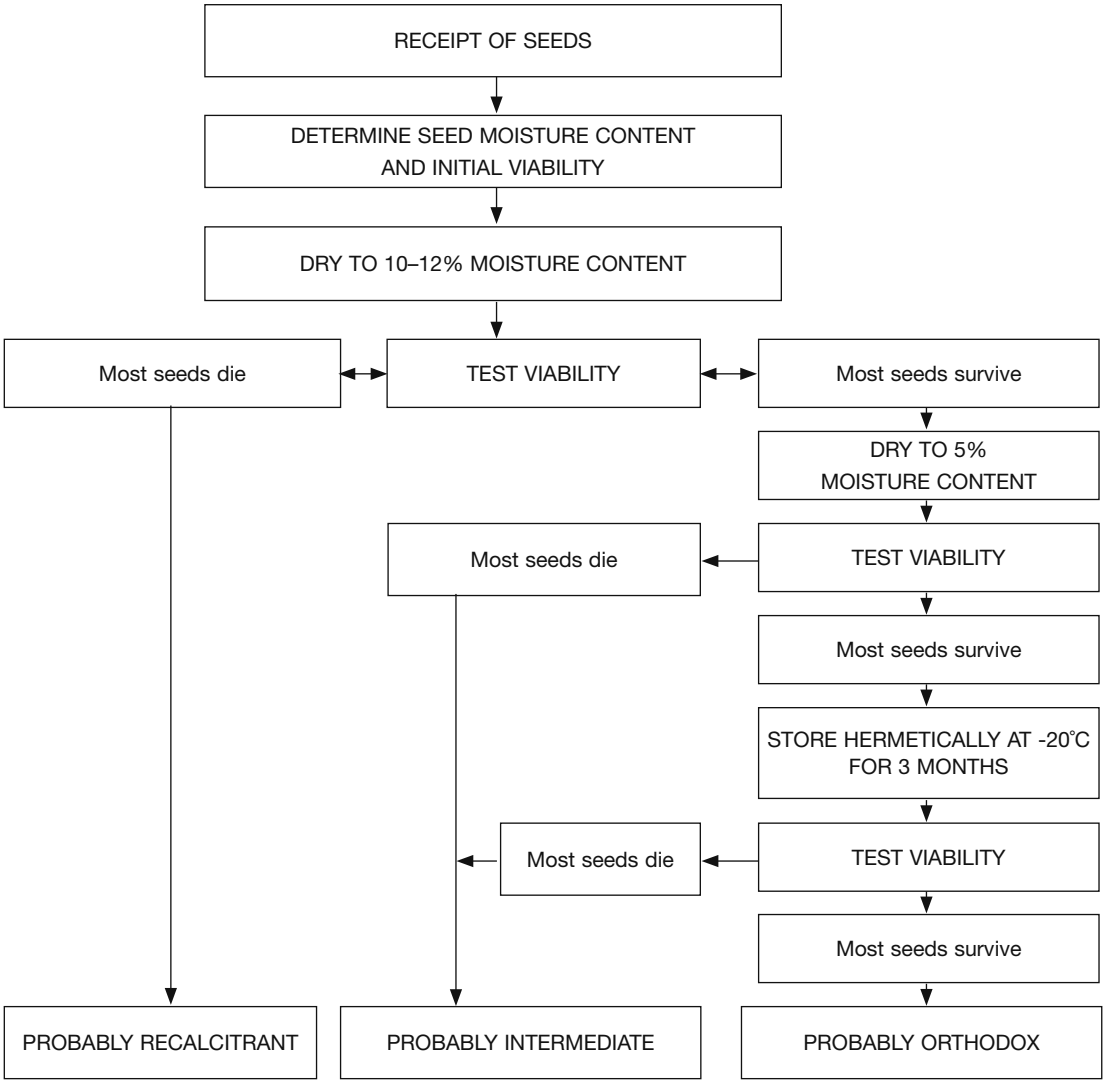
Figure 4.2. Predicting drying time.

high moisture contents. Desiccation sensitivity can be assessed by measuring percentage germination at different intervals of drying (see Flowchart 4.3).

- Seeds that tolerate desiccation (show no loss in viability) to 5% moisture content or below (values in equilibrium with 10–15% RH at 20°C) are likely to show orthodox seed-storage behaviour.
- Seeds that tolerate desiccation to about 10–12% moisture content (values in equilibrium with 40–50% RH at 20°C), but whose viability is reduced when subjected to further desiccation to a lower moisture content are likely to show intermediate seed-storage behaviour.
- Seeds that are killed by desiccation to 15–20% moisture content (values in equilibrium with >70% RH at 20°C) are likely to be recalcitrant.

Information on storage behaviour of a wide range of species is available at www.rbgkew.org.uk/data/sid. A large part of the information included on this website originates from the Compendium on Seed Storage Behaviour by Hong et al. (1996). An electronic version of the compendium database is also available for download from Bioversity's Publications web site: www.bioversityinternational.org/publications/index.asp.

Flowchart 4.3. Protocol to determine seed storage behaviour.



Source: Hong and Ellis (1996).

Seed-drying procedures

Step 1: Predict moisture content and drying period

Assess the need for drying by estimating the moisture content of seeds received at the genebank. A quick measurement of moisture content may be carried out using a calibrated moisture meter as described in section 4.1.

- If the moisture is above the recommended limits for safe storage (3–7% for long-term conservation depending on species), drying is required.

Prediction of drying time

The length of the drying period can be predicted using either of the methods described below. If the genebank has no previous experience with drying seeds of a particular species, it may be necessary to experiment in order to predict the appropriate drying period.

Prediction of drying time by weight loss

1. Determine the moisture content of the seed sample using the methods described in section 4.1.
2. Weigh the seed sample that requires drying.
3. Calculate the weight of the seeds at the required moisture content using the equation:

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

Example:

Initial weight of the seeds = 250 g
Initial moisture content = 12%
Target moisture content at end of drying = 8%
Final weight of seeds

$$\text{at 8\% moisture content} = 250 \times \frac{(100 - 12)}{(100 - 8)} = 239 \text{ g}$$

4. Keep the sample in muslin cloth or a nylon-net bag and allow it to dry, periodically weighing the sample, until the required weight is attained.

Prediction of drying period from mean drying curves

In general, 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 equal under the same environmental conditions. Drying curves can therefore be used for predicting the

drying period of all seed lots of a particular species dried under a given set of conditions. This precludes frequent monitoring of seed moisture content during drying and limits seed waste.

How to prepare mean drying curves

1. Collect 250–500 g of seeds from each of 3–5 accessions (differing in seed characteristics like seed size, mass, shape, chemical composition) of a species. Use seed lots with excess seed or those being discarded due to low viability.
2. Determine the moisture content for each seed lot using the oven-drying method described earlier.
3. Dry the samples using the same method and conditions used in practice.
4. Mix the seeds in a container and remove a small sample each day to determine the moisture content.
5. Repeat daily until no change in moisture content is recorded.
6. Plot the data on a graph with percent moisture content on the Y-axis and drying time on the X-axis.
7. Changes in moisture content over time can be described by fitting an exponential curve (mean drying curve) to the data set.

The mean drying curve can be used as a guide because other seed lots of the same species should dry at a similar rate. This can be repeated with seeds of all species and their drying curves can be plotted for different drying conditions.

Using mean drying curves to predict drying time

1. Use the graph prepared for seeds of a particular species being dried.
2. Determine the initial moisture content of the sample by the oven-drying method.
3. Select the final moisture content that is required for storage of this species.
4. Draw a horizontal line from the initial and desired moisture contents on the vertical Y-axis across to the drying curve.
5. Note the day on the X-axis corresponding to the points of intersection with the drying curve for each of the moisture contents.

The difference between the two points on X-axis indicates the drying time required to achieve the desired moisture content (see Figure 4.2).

Step 2: Prepare seeds for drying

1. It is preferable to place the seeds in porous bags[8] labelled for each accession. When using bags, two labels should always

[8] Bags used for drying should be porous enough to allow moisture to escape easily. Depending on seed size, muslin cloth bags or bags made of mosquito netting are best suited for this purpose.

follow the seed lot—one placed outside the bags and one placed inside with the seeds. Labels should be durable and written with permanent marker.
2. Do not keep a large quantity of seeds in a single bag. Split the accessions into several labelled bags in thin layers to facilitate fast drying.
3. Close the bags properly to ensure no spill-over or mixing of seeds.

Step 3: Dry the seeds

Several methods are available for drying seeds. The most common and safe methods used for drying are *dehumidified drying* and *silica gel drying*. Other methods like *saturated salt solution drying* can also be used.

Avoid using high temperatures for drying, since they will reduce seed storage life.

All these methods rely on leaving seeds in an environment of low RH and allowing the seed moisture content to reach equilibrium at a relatively low temperature (10°–25°C). Note that seeds will reach equilibrium at different rates, depending on species, seed size, and drying conditions. Most seeds will dry quickly at first and the drying rate will slow as low moisture content is approached.

If the initial seed moisture content is too high (>15%), it is recommended that seeds be dried in two stages:
1. initial drying to reduce the moisture content to safe levels in order to avoid rapid desiccation and damage to sensitive seeds (such as cleavage damage in soya bean) (see Box 4.1 for initial drying options); and
2. final drying to moisture content recommended for conservation in genebanks.

Box 4.1. Options for initial drying.

- Outside in shade on open mesh shelves, if the climate is suitable –
  - requires additional control measures against birds, insects and dew
- Passive drying in a room with good ventilation and air circulation –
  - not feasible in hot and humid climates of moist tropics
- Active drying under forced ventilation

Dehumidified drying

This method involves drying seeds in an environment where RH is kept low by use of dehumidifiers. The FAO/IPGRI Genebank Standards (1994) recommend a range of 10–15% RH and a temperature of 10°–25°C for drying seeds. For smaller genebanks, seed-drying cabinets designed to provide these conditions are available. Larger genebanks may require modular walk-in seed-drying rooms. The drying cabinet or room should have a safety

device to regulate temperature and prevent overheating in the event of mechanical failure.

1. Place the seeds, which have been packaged in the cloth bags, on the open racks of a drying room or seed-drying cabinet. Make sure that the seed bags are not stacked too closely and that there is enough space to allow the free circulation of air between them.
2. Leave the seeds in the drying room or seed-drying cabinet until the moisture content is likely to be in the range required for storage. If the initial moisture content and weight of the sample are known, the length of drying period can be predicted by using mean drying curves or by measuring weight loss as described above (see step 1).
3. Alternatively, remove a sub-sample and determine whether or not the required moisture content has been attained using the methods described in section 4.1.

Silica gel-drying

Small samples can be dried using silica gel. The procedure for drying seeds using blue silica gel is explained below.

1. Place dried self-indicating blue silica gel[9] in a desiccator or glass jar with an airtight seal. The weight of the silica gel used should be equal to that of the seeds for efficient drying. For faster drying, some genebanks use higher gel-to-seed ratios such as 3:1.
2. Place the seeds in porous bags and keep them in close proximity to the silica gel.
3. Keep the desiccator at a cool temperature (approximately 20°C).
4. Change the silica gel daily or when the colour changes from deep blue to pink or pale blue.
5. Regenerate the silica gel by heating it at 100°C until it turns deep blue again. Allow it to cool in an airtight container before reusing.
6. Leave the seeds with fresh silica gel in the container until the moisture content of the seeds is in the range required for storage.
7. Pack the seeds in appropriate containers once the recommended moisture content or equilibrium seed weight is attained, and when the germination level and seed health are acceptable.

Calcium chloride drying

Seeds can also be dried using anhydrous calcium chloride granules. Calcium chloride is safe, non-toxic and inexpensive. It is readily available at hardware stores and easily disposed of by washing it down the drain. The drying method is very similar to that for silica

[9] Users of the traditional self-indicating blue silica gel are strongly cautioned regarding the possible carcinogenic effects of cobalt chloride, which is used as the indicator. The gel should be handled in a fume cupboard whenever there is a risk of generating dust. Alternatives to blue silica gel such as the orange-to-colourless granular self-indicating silica gel or the less dusty beaded silica gel (2-5 mm) are available from most laboratory chemical suppliers and should be used when possible.

gel, but the chemical is disposed of after drying or can be re-used for making saturated salt solutions.

1. Place anhydrous calcium chloride granules in a desiccators or glass jar with an airtight seal and a wire mesh shelf above the chemical. Close the container quickly to avoid absorption of moisture from the air.
2. Place the seeds in porous bags on the desiccator plate or a wire mesh.
3. Keep the desiccator at a cool temperature (approximately 20°C).
4. When the top layer of calcium chloride becomes hard and shiny, turn it over so that the bottom part is at the top. Once it becomes completely hard, it can be re-used for making a saturated salt solution as described further below.
5. Leave the seeds with fresh calcium chloride in the container until the moisture content of the seeds is in the range required for storage.
6. Pack the seeds in appropriate containers once the recommended moisture content or equilibrium seed weight is attained, and if the germination and seed health are acceptable.

Saturated salt solutions

Seeds can be prepared for storage by drying them in sealed containers over saturated solutions of mineral salts such as calcium chloride and lithium chloride. Calcium chloride maintains a RH of 30% at 25°C and can be used to dry seeds for medium-term conservation. Similarly, lithium chloride provides 13% and calcium bromide 18% RH at 20°C, and can be used to dry seeds for long-term conservation. Mixtures of calcium chloride with lithium chloride can also be used to reach lower seed moisture contents at a lower cost than lithium chloride alone. The exact RH and targeted moisture content must be determined for the specific ratio of chemicals used.

To prepare the salt mixture:

1. Mix the salt with water to form a wet slurry.
2. Place the slurry in a desiccator or an open container and place the container into a larger airtight container that will be used to dry the seeds.
3. Spread the seeds in a thin layer inside their container and place it in the desiccator or the larger container. The salt mixture should not come into contact with the seeds. Seal the lid on the larger container with the seeds and the slurry.
4. Allow enough time for seed moisture to reach equilibrium with the air inside the container—this may take several weeks. Circulating the air inside the container will speed the drying process.

Other low-cost methods

Self-defrosting refrigerator

If mineral salts are not available, seeds can be dried using a self-defrosting refrigerator. The action of the self-defrost unit will maintain a low RH inside the refrigerator. It is difficult to control the exact RH, but this method is satisfactory if better means are not available. The RH in many refrigerators ranges from 10–40%, corresponding to seed moisture contents suitable for long- or medium-term conservation.

1. Spread seeds in a thin layer in an open container.
2. Place the container in a self-defrosting refrigerator and allow seeds to reach equilibrium with the humidity inside the refrigerator.
3. Seal the drying container tightly, remove it from the refrigerator and allow it to reach room temperature before opening it to prevent moisture from condensing on the seeds.
4. Seal the seeds in airtight containers and transfer them to storage.

Shade-drying

Shade-drying can be an effective way of reducing seed moisture content in environments where the RH is low (less than 40%); the lower the humidity, the more effective the drying process will be. Shade-drying is particularly useful for initial drying. Do not dry in the sun because it is believed to affect long-term seed viability in some species.

1. Lay seeds in a single layer on a linen sheet or on open mesh racks placed in the shade, ensuring the free circulation of air. Any device that can increase the flow of air over the seeds (such as a fan) will improve drying efficiency.
2. Cover seeds with a protective net to prevent predation by animals (birds, rats, etc.).
3. At night, wrap the linen sheet and keep it in a cool room.
4. Allow enough time for seed moisture to reach equilibrium with the ambient RH—this may take several days.

In tropical countries with high RH, it is more difficult and expensive to maintain a drying room at very low RH. A combination of methods including low-cost technologies such as silica gel-drying and saturated salts can be used to effectively reduce seed moisture contents to accepted levels.

Documentation

The following descriptors can be used to document information regarding moisture content determination and seed-drying procedures for individual accessions:

- Seed moisture content at the time of receipt (%)
- Method used for moisture determination
- Pre-drying method, duration (where necessary)
- Final drying method

- Duration of final drying
- Final moisture content after drying (%)
- Date of final moisture content determination
- 100- or 1000-seed weight (g)
- Total dry weight of seeds (g)

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