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INTERNATIONAL BOARD FOR PLANT GENETIC RESOURCES

PRACTICAL MANUALS FOR GENEBANKS: NO. 1

PROCEDURES FOR HANDLING SEEDS IN GENEBANKS

by

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PREFACE

This manual is the first in a series of practical guides to suitable methods for processing and storing seeds in genebanks. It has been prepared specifically for genebank personnel, especially those technicians and staff involved in day-to-day seed handling. The manual attempts to explain step-by-step the practical procedures used in handling seeds in genebanks, especially those holding base collections. A series of scientific standards for the correct conservation of both base and active collections have been defined by the IBPGR Advisory Committee on Seed Storage. The techniques described in this manual are compatible with procedures recommended by the Committee. The operating procedures of genebanks must meet the high standards necessary for adequate conservation of germplasm, otherwise valuable materials will be lost - often irretrievably.

The techniques outlined here have been tried out and tested in genebanks; however, it should be realized that the techniques described here are not the only methods available. Seed technologists use many different techniques, but those selected and described in this manual are considered the most suitable for handling seeds of variable character within the same sample or accession. It is hoped that they form a useful basic guide.

Further detailed information of the techniques described in this manual and of alternative techniques can be found in the publications listed in Appendix I. In particular more detailed scientific information for curators can be found in the IBPGR publication 'Handbook of Seed Technology for Genebanks' by Ellis, Hong and Roberts (1985). Further advice can also be sought from the IBPGR Seed Conservation Officer, the IBPGR Information Officer and from the members of the IBPGR Advisory Committee on Seed Storage, whose names and addresses are found in Appendix IV.

The author would like to acknowledge the helpful and practical comments made by D. Astley, R.H. Ellis, S. Linington, E.H. Roberts, R.D. Smith, K.L. Tao and J.T. Williams during the preparation of this manual.

Introduction

Genebank Operations

The operation of a genebank is a complex activity which must be carried out in an orderly and efficient way to adequately conserve the genetic resources that it holds in care. In view of the responsibilities involved in curating these resources, the standard of day-to-day work must be careful and precise. There are two major categories of genebanks:

1. Base collections store accessions under adequate conditions for long-term storage. They are not used as a routine source for distribution of germplasm but as a security against loss. Seeds are removed from base collections at infrequent intervals to monitor viability and for regeneration, when seed viability drops or when stocks of an accession are no longer available.

2. Active collections store accessions from which seed samples are drawn for distribution, regeneration and evaluation of plant characteristics. Storage conditions are usually less demanding than those of base collections because seeds are stored for shorter periods (medium-term).

Base and active collections are closely linked. All the accessions stored in base collections should also be available from several active collections. Base and active collections need not be located in the same place, although they often are. Information about the accessions must flow freely between the different genebanks.

Many diverse tasks are involved in conserving germplasm in the best possible way. The major activities and procedures involved are the same for both base and active collections. They are explained in detail in the following Sections:

I.	Sample registration
II.	Seed cleaning
III.	Seed moisture content
IV.	Seed drying
V.	Seed viability
VI.	Seed packaging
VII.	Seed storage
VIII.	Monitoring accessions
IX.	Distribution of germplasm from active collections
X.	Regeneration of accessions

General Workplan

When seed samples are received and are entered into the genebank, there is a general scheme which can be followed so that the seeds are processed and entered into the genebank with speed, accuracy and efficiency. The flow chart in Figure 1. shows this general scheme and the preferred order which the procedures should follow. A typical workplan would be:

* Seeds are received at the genebank. They are checked and registered and sent for cleaning.

* The seeds are first checked for insect infestation and purity. If there is no sign of insect damage the seeds can be sent directly for cleaning and drying. If insects are present the seeds should be isolated and sent directly for seed drying using silica gel in separate containers to prevent the spread of insects as outlined in Section II.

* Samples with few seeds will need regeneration to provide sufficient seeds for storage. Regeneration of a sub-sample of the accession should be done as soon as possible, but seeds should be dried and stored temporarily at low temperatures.

* Drying is continued over several days or weeks. When it is predicted that the seed moisture content has reached between 3 - 7%, sub-samples are tested to accurately determine the moisture content using the techniques outlined in Section III.

* Sub-samples are also taken to determine seed viability as explained in Section V after the drying period, so that the results are available before the seeds are packaged. Poor quality seeds will be found at this time and arrangements can be made for a sub-sample of the accession to be regenerated and good quality seeds placed into the genebank. Seeds awaiting regeneration before final storage should also be stored temporarily in good storage conditions, but should not be a part of the genebank distribution list.

* Seeds which are dry are packaged as explained in Section VI.

* The seeds are placed into the seed store and the data recorded in the inventory, together with the data about seed viability when the data becomes available.

* At regular intervals after storage, the seeds are monitored for viability as explained in Section VIII. When the viability has fallen below 85%, the accession should be regenerated and fresh seeds placed in the genebank.

* The seeds are distributed on request from active collections and the data recorded in the appropriate data files as outlined in Section IX.

* When the number of seeds in store falls to a low level, the seeds will need to be regenerated as outlined in Section X.

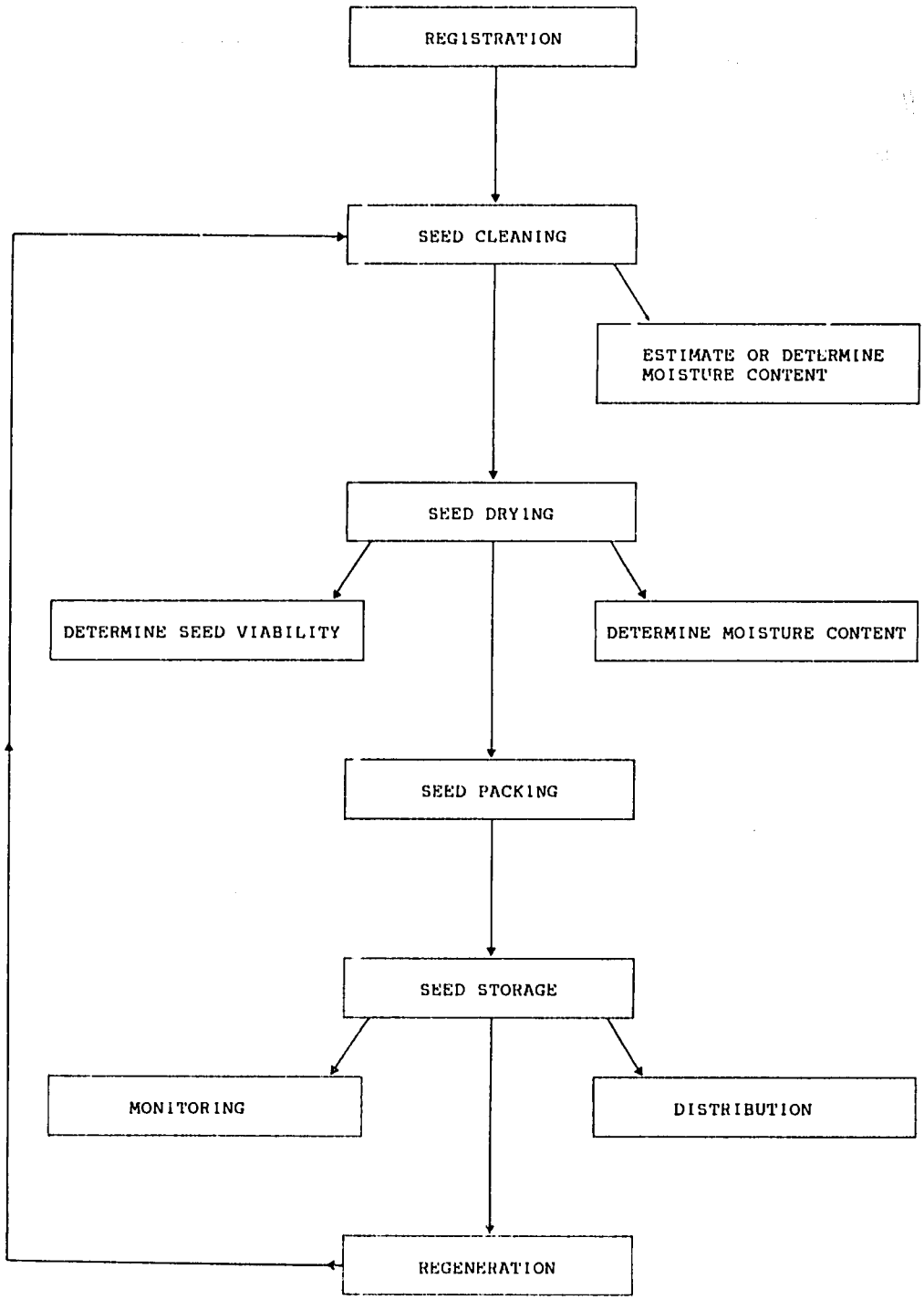


Figure 1. Preferred sequence of events in a genebank.

General Rules

* Process the seeds as quickly as possible.

  Why? Because seeds left around in the open may become infested with insects, will lose viability and can easily become mixed.

* Dry seeds as soon after receipt as possible.

  Why? Because drying seeds to low moisture contents will help to improve their storage life even at room temperatures.

* Never place wet seeds in sub-zero temperatures.

  Why? Because seeds with moisture contents of approximately 16 to 20 percent or more can show cellular damage during freezing which causes loss of viability.

* Once seeds have been dried, store them in moisture-proof containers.

  Why? Because the moisture content of seeds will always equilibrate with the relative humidity of the surrounding air and dried seeds will absorb moisture again, unless properly protected from the air.

* Treat all accessions separately.

  Why? Because different accessions of the same species can have different storage characteristics.

* Keep all the data relating to an accession.

  Why? Because the more information available about an accession the more useful it is and information which might seem irrelevant may be important in the future.

* Always work in an orderly and accurate way.

Daily Procedures

Some tasks should be done every day to make sure that the genebank runs efficiently. These are:

TASK 1. KEEP A DAILY RECORD OF WORK DONE

1. Record the daily work in a record book or desk diary. This will enable you to check back for the date when samples were received and when they were tested.

2. Use this book to write down the date of any future tasks as a reminder.

TASK 2. KEEP A DAILY RECORD OF THE ENVIRONMENTAL DATA OF THE FACILITY

1. Measure and record the maximum and minimum temperatures in the laboratory, drying room and seed storage room(s) or freezers. Keep these data in either a log book or a data file.

2. Measure and record the relative humidity in the laboratory, drying room and seed storage room(s) at a cool time and at the hottest part of the day.

TASK 3. KEEP COMPREHENSIVE DATA FILES ON THE SEED ACCESSIONS

1. Data files can be in the form of notebooks, filing cards and computer files.

2. Use different files to record the different data types:

Management data files	to record data concerned with the management of the accessions and the seed store.
Passport data files	to record passport and collection data.
Characterization and evaluation data files	to record data collected during the characterization and evaluation of the accessions.

General work plan for your genebank

Outline the general work plan used in your genebank:

SECTION I

Sample Registration

What is sample registration?

This is the registration of the sample into the genebank and the recording of the identification data into the data files relevant to each seed sample or accession.

Why is it necessary?

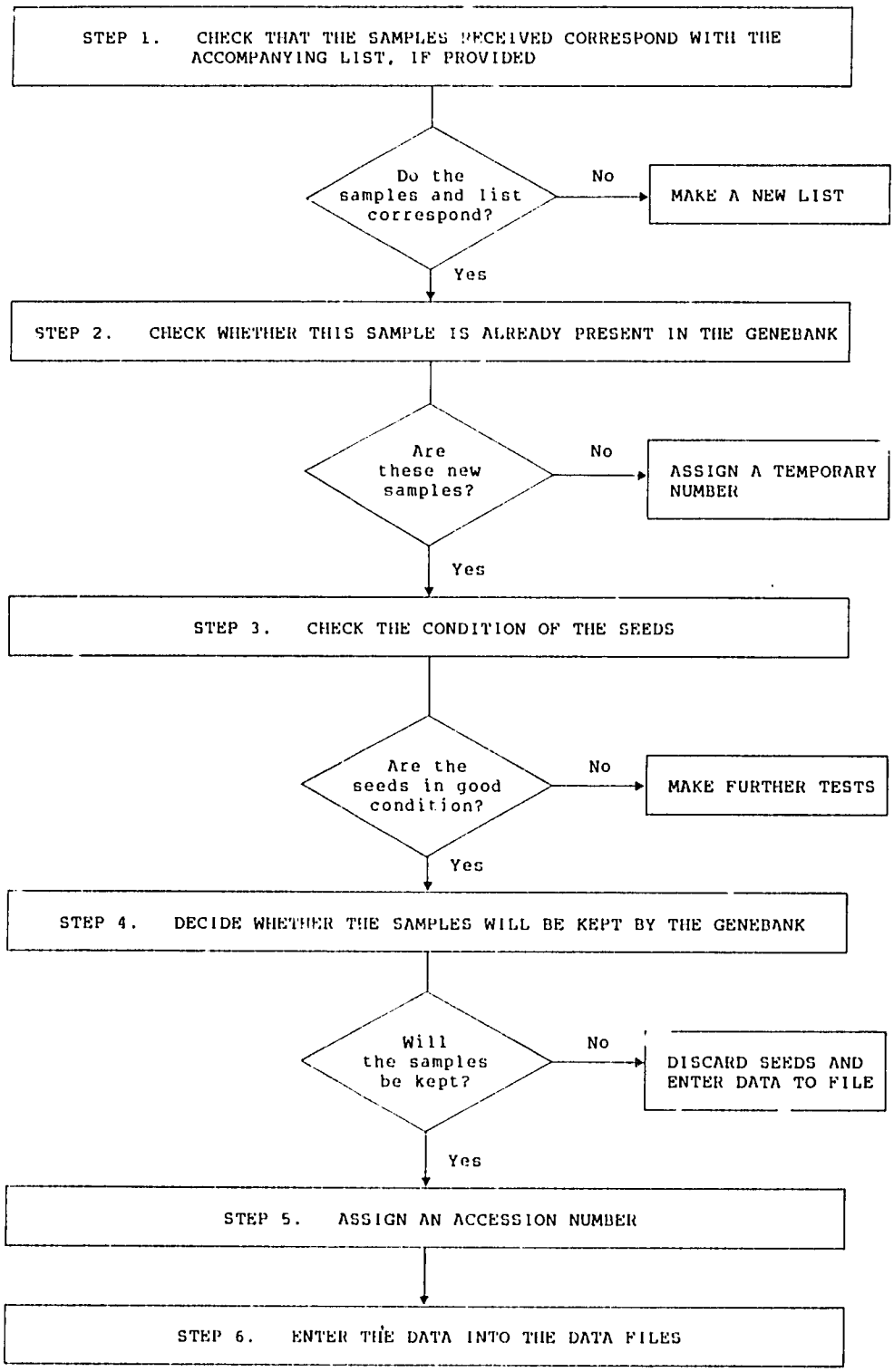
Registration is carried out to allow you to keep a record of which samples are held in the genebank and to produce inventory lists of samples which are available for distribution.

When is it done?

The samples must be registered when they first enter the genebank; even before they are processed. If final registration cannot be completed at this time, due to the need for tests or checking records, then a temporary number can be assigned until such time as the sample is ready for a permanent accession number.

How is it done?

The best way to register samples is to assign each one a unique sample accession number in the genebank and record all the data relating to each sample in a set of files. A simple method for registration of samples is outlined in the following pages.


STEP 1. CHECK THAT THE SAMPLES RECEIVED CORRESPOND WITH THE ACCOMPANYING LIST, IF PROVIDED
Do the samples and list correspond?
No
MAKE A NEW LIST
Yes
STEP 2. CHECK WHETHER THIS SAMPLE IS ALREADY PRESENT IN THE GENEBANK
Are these new samples?
No
ASSIGN A TEMPORARY NUMBER
Yes
STEP 3. CHECK THE CONDITION OF THE SEEDS
Are the seeds in good condition?
No
MAKE FURTHER TESTS
Yes
STEP 4. DECIDE WHETHER THE SAMPLES WILL BE KEPT BY THE GENEBANK
Will the samples be kept?
No
DISCARD SEEDS AND ENTER DATA TO FILE
Yes
STEP 5. ASSIGN AN ACCESSION NUMBER
STEP 6. ENTER THE DATA INTO THE DATA FILES

STEP 1. CHECK THAT THE SAMPLES RECEIVED CORRESPOND WITH THE ACCOMPANYING LIST, IF PROVIDED

1. Arrange the packets containing the seed samples in alphabetical or numeric order depending on the identification provided on the packets.

2. Check all the packets against the list provided with the samples.

3. If no list was provided or the seeds do not correspond with the list, prepare a new list and check again that all packets have been included. Write to the donor asking for the original list.

4. Acknowledge receipt of the seeds to the donor.

STEP 2. CHECK WHETHER THIS SAMPLE IS ALREADY PRESENT IN THE GENEBANK

1. Look in the records of passport data.

2. Check the collector's number to see if this sample has already been sent to the genebank from another source.

STEP 3. CHECK THE CONDITION OF THE SEEDS

1. Open each packet of seeds and check their condition by looking for obviously poor seeds which probably would not germinate.

2. Observe whether there is any insect damage, fungal growth, damaged, broken, empty or shrivelled seeds.

3. If no phytosanitary certificate accompanies seeds from outside your country or there is any doubt about the health of the seeds, contact the quarantine officials for advice.

STEP 4. DECIDE WHETHER THE SAMPLES WILL BE KEPT BY THE GENEBANK

1. If the seeds are in good condition and are not already included in the genebank, each sample should be assigned an accession number (follow the system used in your genebank) as described below.

2. If the seeds are in poor condition or are definitely already in the genebank in sufficient quantities, check with the curator that they can be discarded or given to another institute for use there.

3. If in doubt assign a temporary number or code to identify the sample following the system used in your genebank, until further checks can be made.

4. When all these further checks have been made, a decision about whether to keep each sample will be made by the curator. If the sample is to be kept, assign an accession number as described below. If the seeds are dead or the sample is already represented in the genebank, discard the material and the temporary number and make a note in the files of the action taken and reason for it.

STEP 5. ASSIGN AN ACCESSION NUMBER

1. Check in the data files to determine which was the last accession number used for a sample.

2. Give the next ascending accession number to the first sample on the list and consecutive numbers to each sample.

Notes and Examples

Equipment

Notebooks
Permanent markers
Pencils

Arrange the seed packets, by either genus, species, collector's name or ascending number.

Example:

Cajanus cajan

PL0785, PL0789, PL1001, TM0005, TM0098

Desmodium sp.

PL0654, PL0786, TM0097, TM0567, WS0004

The easiest way to check whether a sample is in the genebank is to check the collector's number. This is unique and should always remain with the passport data. It can easily be checked to see if the same sample has arrived from another source.

Look carefully for signs of insects such as small holes, eggs or feeding damage and for fungal hyphae. Check that the seeds are full and in fact seeds and not empty fruits. Some species are noted especially for having empty seeds or fruits.

Make sure that temporary numbers are easily distinguished from accession numbers.

3. Write the accession number clearly on the seed packet using a permanent marker, on the list of new samples and in the data files of the genebank.

STEP 6. ENTER THE DATA INTO THE DATA FILES

1. For each accession, record all the passport and original identification data in the designated fields (descriptors) of the passport data file.

2. When data are missing leave the field blank and try to fill in the data at a later date when more information is available. Write to the donor asking him to supply the missing data.

Notes and Examples	Equipment
Data files stored on paper (in books or on file cards) are suitable for use with small numbers of accessions and are often more accessible to all staff. File cards are better than books because they can be sorted and rearranged as required but single cards are more easily lost. Computers are useful for genebanks with large amounts of data and many accessions. Make sure your files are kept in a clear orderly manner and as up-to-date as possible.	Computer and programmes File cards

Notes and comments on sample registration in your genebank

Use this page to make notes for future reference:

SECTION II

Seed Cleaning

What is seed cleaning?

This is the removal of any debris or low quality, infested or infected seeds and seeds of different species (weeds) that are foreign to the sample.

Why is seed cleaning important?

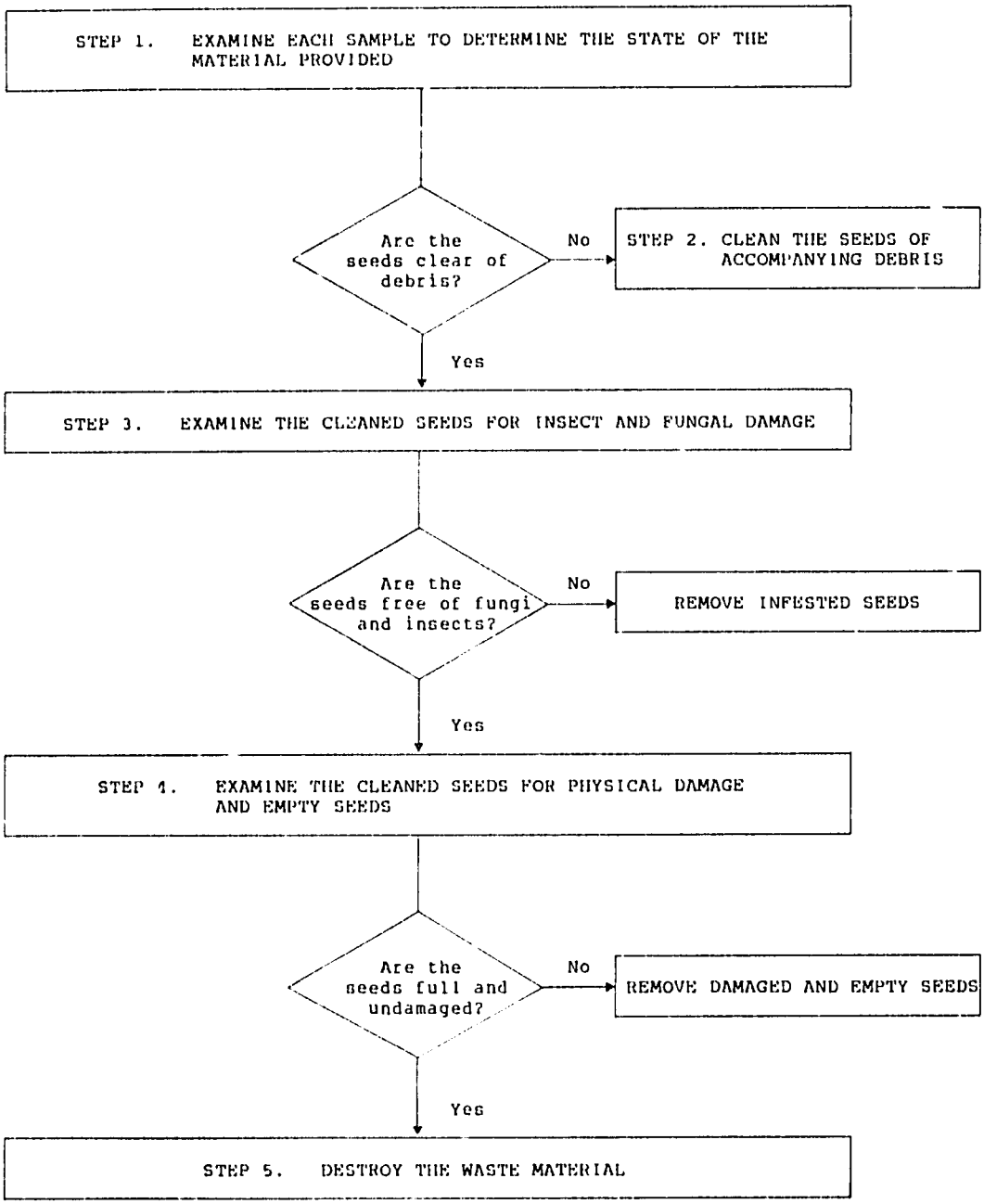
The cost of maintaining an accession in a genebank is high and space is limited. Debris and damaged seeds can spread infection. Therefore only good quality viable seeds should occupy space in the store and damaged or non-viable seeds should be destroyed to prevent the spread of infection. Each accession should be represented in the genebank by a minimum number of clean seeds. FOR INFORMATION ON SAMPLE SIZE, SEE SECTION VII (PAGE 78).

When should seed cleaning be carried out?

Seeds should be cleaned immediately after registration or harvest. If the seeds are very moist and cleaning is done by machines, it may be necessary to dry the seeds within the fruits to within 12 - 16% moisture content before cleaning to prevent damage. Seeds donated by other institutes often arrive already cleaned.

How should cleaning be done?

Cleaning should be done in a way that causes the least damage to the sample and does not waste good seeds. The following procedures can be used as a guide.


STEP 1. EXAMINE EACH SAMPLE TO DETERMINE THE STATE OF THE MATERIAL PROVIDED
Are the seeds clear of debris?
No
STEP 2. CLEAN THE SEEDS OF ACCOMPANYING DEBRIS
Yes
STEP 3. EXAMINE THE CLEANED SEEDS FOR INSECT AND FUNGAL DAMAGE
Are the seeds free of fungi and insects?
No
REMOVE INFESTED SEEDS
Yes
STEP 4. EXAMINE THE CLEANED SEEDS FOR PHYSICAL DAMAGE AND EMPTY SEEDS
Are the seeds full and undamaged?
No
REMOVE DAMAGED AND EMPTY SEEDS
Yes
STEP 5. DESTROY THE WASTE MATERIAL

STEP 1. EXAMINE EACH SAMPLE TO DETERMINE THE STATE OF THE MATERIAL PROVIDED

1. Treat each accession separately.

2. Empty the contents of each packet into a labelled tray large enough for the material to be spread out.

3. Check whether the seeds are in dry fruits or fleshy fruits or have already been cleaned.

STEP 2. CLEAN THE SEEDS OF ACCOMPANYING DEBRIS

Seeds from dry fruits

1. Check that the seeds are dry enough to be cleaned without damage because moist seeds (above 16% moisture content) can be damaged during cleaning.

2. If the samples are very moist, place the container with the fruits in the drying room, if available, or in a shady place to dry.

3. When it is predicted that the seed moisture content is in the range of 12 - 16%, the seeds can be safely cleaned.

4. Clean the seeds from the fruits, preferably by hand.

5. Small threshing machines can be used for some crops, provided that they do not damage the seeds. Clean threshing machines between each accession to prevent mixing of seeds from different accessions. Mechanical cleaning is of less value in multi-crop genebanks.

6. Remove debris from the seeds by the use of gradated sieves.

7. Pieces of debris of a similar weight and size as the seeds will have to be removed by hand.

8. Separate dry lightweight material from the seeds by winnowing, blowing gently on the seeds or by use of a seed blower.

9. Return the cleaned seeds to the tray ready for inspection.

Seeds from fleshy fruits

1. Some species have fruits which irritate the skin, e.g. Solanaceae, and gloves should be worn when handling them.

2. Open the fruit using a knife but take care not to cut through the seeds.

3. Extract the seeds from each fruit and place in a tray.

4. Remove any juice or flesh adhering to the seeds using clean cold water and a small brush to remove tenacious flesh.

5. If mucilage surrounds the seeds and cannot be removed by washing in clean water, gently rub the wet seeds with coarse sand and then wash off the sand and mucilage in a sieve. It is also possible to dry the seeds and then rub the dry mucilage off, but the seeds must be well separated, to prevent their sticking together during drying, on a surface to which they will also not stick.

Notes and Examples	Equipment
Seeds which are moist can be bruised and damaged during extraction. Seeds which are too dry are brittle and can easily crack. Cleaning by hand is gentler and will do less damage to the seeds.	Small containers and trays Set of sieves Bamboo baskets Paper packets Threshing machine Seed blower Spatulas
With small samples it is more convenient and quicker to clean by hand because of the time needed to clean machinery between accessions. The flat bamboo baskets used in many countries are ideal for cleaning and winnowing.	
Use your knowledge of the morphology of the fruit and the position of the seeds to ensure that seeds are not damaged by cutting. It is often extremely difficult to remove flesh from small seeds. It may be easier to dry the seeds and rub off the flesh when dry.	Plastic bowls Nail brush Absorbent paper Paper packets Spatulas

6. Do not allow seeds to remain in the water too long because they will absorb moisture which can cause the start of germination and then cause a loss of viability when the seeds are dried.

7. Dry the seeds in a thin layer, preferably one seed thick, on cotton towelling or paper in the drying room, if available, or in a cool shady place.

STEP 3. EXAMINE THE CLEANED SEEDS FOR INSECT AND FUNGAL DAMAGE

1. If the seeds are infested with insects or infected with fungi, isolate the sample and dry the infested seeds in sealed containers with silica gel to prevent the spread of the insect.

2. It is a general rule that drying seeds to a low moisture content will prevent further growth of fungi and development of further reproductive cycles of insects, but it will not kill them.

3. Storage at sub-zero temperatures will kill the adult insects but not fungi. Larva and eggs are more difficult to kill.

4. If seeds are to be stored above 0°C, place in a freezer for about 7 days to kill the insects before continuing with the normal packaging and storage procedures.

STEP 4. EXAMINE THE CLEANED SEEDS FOR PHYSICAL DAMAGE AND EMPTY SEEDS

1. Spread the seeds on a flat well lit surface of contrasting colour. An illuminated table can be used if available.

2. Examine the seeds carefully for physical damage and empty seeds.

3. Discard any visually damaged, shrivelled or empty seeds.

4. Empty seeds may be difficult to detect unless the seeds are dissected or tested with special X-ray equipment to determine the percentage of empty seeds (Ch. 8, Ellis, Hong and Roberts, 1985).

5. Empty seeds are lighter than full ones and can be separated by use of the seed blower.

STEP 5. DESTROY THE WASTE MATERIAL

1. Destroy any waste material to prevent spread of the disease or insects to other material.

Notes and Examples	Equipment
Make sure that the paper or cotton towelling used is clean to prevent contamination and avoid affecting the subsequent germination.	
The use of low temperatures prevents fungal growth, but the spores remain viable during storage and can begin to grow again when the seeds are sown or germinated. To avoid this, fungicides can be used at planting but should not be used during storage because some chemicals can reduce seed longevity over long periods.	
Effective treatment to kill insects without the use of insecticides which may damage seeds during storage is also possible. Seed drying prevents further development and sub-zero temperatures for about 7 days will kill the insects and larvae. Seeds must be dry or viability will be lost by treatment at these temperatures. Large seed or seeds packaged in bulk may take longer to treat to ensure that the centres of the seeds have been exposed to the low temperature.	Freezer
	Seed blower Light table Scalpels Forceps Spatulas
Take care that lightweight full seeds are not discarded during this process. If in doubt dissect some of lighter seeds taken from the debris to check whether they contain embryos.	
If possible burn the waste material to ensure complete destruction.	

Table of standard cleaning procedures for use in your genebank

Fill in this table to use for your future reference:

Species	Method
.....	.....
.....	.....
.....	.....
.....	.....
.....	.....
.....	.....
.....	.....
.....	.....
.....	.....

SECTION III

Seed Moisture Content

What is seed moisture content?

The moisture content is the amount of water in the seed and is usually expressed as a percentage. It can be expressed on either a wet weight basis (where it is expressed as a percentage of the fresh weight of the seed) or on a dry weight basis (where it is expressed as a percentage of the dry weight of the seed). For genebank work it is usually expressed on a wet weight basis and all determinations and calculations should follow this rule.

The seed moisture content can either be accurately determined experimentally by scientific techniques, or it can be predicted approximately from the information available. The determination is destructive to the seeds used and since in many cases a prediction is sufficient, determination should only be carried out where essential.

Why is it important to determine moisture content?

A small change in seed moisture content has a large effect on the storage life of the seeds. Therefore it is important to know the moisture content in order to make a reasonably accurate prediction of the possible storage life of each accession.

When should moisture content be determined and when should it be predicted?

Accurate determinations should be kept to a minimum because for many activities a prediction is sufficient. In most cases this means one accurate determination after the drying period, so that the moisture content of the stored seeds is known and can be used to make better predictions of storage life.

How should moisture content be determined and how should it be predicted?

The methods outlined in the following pages can be used as a guide to prediction but experience in dealing with a crop in your own genebank will be necessary for reasonable predictions to be made. The methods for determination are modified from those of the International Seed Testing Association (ISTA) as recommended by the IBPGR Advisory Committee on Seed Storage. Other methods are available and can also be used provided that the results give an accurate determination of moisture content and are calculated on a wet weight basis, to enable comparisons to be made between materials and between genebanks.

Prediction of seed moisture content

The possibility of making reasonably accurate predictions of moisture content will improve as you gain experience with seeds of different species and use your knowledge of the origin of the seeds and the conditions in which they have been stored. The moisture content of a seed will equilibrate with the relative humidity of the air surrounding it. This moisture content is called the equilibrium moisture content and because it is constant for any species at known temperature and relative humidity, it can be used as an approximation of the actual seed moisture content.

Moisture contents will differ from seed to seed within the same accession, but should not be sufficiently different to make more than about 1% difference in moisture contents between seeds of the same accession and different accessions of the same species. This is sufficiently close for a prediction.

A list of a few common species and their equilibrium moisture contents is provided for guidance (Table 1). Further information is available from Cromarty, Ellis and Roberts (1982). For species not on the list, it is possible to experimentally determine equilibrium moisture contents for these species at the temperatures and relative humidities at which you are working and prepare your own table to use for future predictions.

Determination of seed equilibrium moisture content

1. Do not waste seeds of germplasm for this, but use either excess seeds or those which are being discarded because they have lost viability.

2. Take two lots of seeds of the species of known weight and leave them in open containers in the environment of your genebank to equilibrate. This may be several days for small seeds and longer for large seeds. Seeds will have reached equilibrium with the moisture content of the air when there is no change in weight on different days.

3. Measure the relative humidity of the atmosphere where the seeds have been held for the past few days by using an aspirated hygrometer.

4. Measure the mean temperature.

5. Remove a sample of seeds and do an accurate determination of moisture content for each seed lot as described on page 32.

6. The mean of the two tests can be used as a guide because other seed lots of the same species should equilibrate at a similar moisture content under these conditions.

7. Repeat the determination the following day to make sure that the moisture content is stable and that the equilibrium has been reached.

8. The work can be repeated using a number of species which are of interest at a range of relative humidities that are common in your country at different times of the year.

9. Make a table of the equilibrium moisture contents at different temperatures and relative humidities and use this to refer to in the future.

Table 1. Equilibrium moisture contents of some common crop seeds at 25°C 1/

Species	Percentage relative humidity					
	15	30	45	60	75	90
Barley (Hordeum)	6.0	8.4	10.0	12.1	14.4	19.5
Beet (Beta)		5.8	7.6	9.4	11.2	
Buckwheat (Fagopyrum)	6.7	9.1	10.8	12.7	15.0	19.1
Cabbage (Brassica)		5.4	6.4	7.6	9.6	
Carrot (Daucus)		6.8	7.9	9.2	11.6	
Cucumber (Cucumis)		5.6	7.1	8.4	10.1	
Egg plant (Solanum)		6.3	8.0	9.8	11.9	
Flax (Linum)	4.4	5.6	6.3	7.9	10.0	15.2
Groundnut (Arachis)	2.6	4.2	5.6		9.8	13.0
Lettuce (Lactuca)		5.1	5.9	7.1	9.6	
Lima bean (Phaseolus)		7.7	9.2	11.0	13.8	
Maize (Zea)	6.6	8.4	10.2	12.7	14.4	18.8
Mustard (Brassica)		4.6	6.3	7.8	9.4	
Oat (Avena)	5.7	8.0	9.6	11.8	13.8	18.5
Okra (Abelmoschus)		8.3	10.0	11.2	13.1	
Onion (Allium)		8.0	9.5	11.2	13.4	
Radish (Raphanus)		5.1	6.8	8.3	10.2	
Rice (Oryza)	5.6	7.9	9.8	11.8	14.0	17.6
Rye (Secale)	7.0	8.7	10.5	12.2	14.8	20.6
Sorghum (Sorghum)	6.4	8.6	10.5	12.0	15.2	18.8
Soyabean (Glycine)	4.3	6.5	7.4	9.3	13.1	18.8
Tomato (Lycopersicon)		6.3	7.8	9.2	11.1	
Turnip (Brassica)		5.1	6.3	7.4	9.0	
Watermelon (Citrullus)		5.1	6.3	7.4	9.0	
Wheat (Triticum)	6.5	8.5	10.4	12.1	14.6	19.8
Winter squash (Cucurbita)		5.6	7.4	9.0	10.8	

1/ Information derived from:
Roberts, E.H. (ed.) (1972) Seed Viability. Chapman and Hall, London.

Table of equilibrium moisture contents of seeds stored in your genebank

Do the experimental work and fill in this table to use as your future guide:

Species	Temperature	Percentage relative humidity	Seed moisture content
.....			
.....			
.....			
.....			
.....			
.....			
.....			
.....			
.....			

How to predict seed moisture content

STEP 1. MEASURE ENVIRONMENTAL CONDITIONS

1. Measure the relative humidity of the atmosphere where the seeds have been held for the past few days by using an aspirated hygrometer.

2. Measure the mean temperature where the seeds have been held for the past few days.

3. If the seeds are from a different environment, they must be allowed to equilibrate with the current one before any predictions are made.

STEP 2. CHECK THE EQUILIBRIUM MOISTURE CONTENT IN THE TABLE

1. Find the name of the crop in the left hand column of the tables of equilibrium moisture contents on pages 26 and 27.

2. Read off the equilibrium seed moisture content at the relative humidity measured in Step 1 above.

3. Use this figure of approximate moisture content as your predicted value in making estimates of drying period or when an exact value of moisture content is not necessary.

Notes and Examples

Wet and dry bulb thermometers can also be used to measure relative humidity. They do not give as accurate a result because the air around the bulbs is static.

Allow sufficient time for the seeds to reach equilibrium. Large seeds take longer to equilibrate.

Equipment

Aspirated hygrometer
Maximum and minimum thermometer

Determination of seed moisture content

Seed moisture content can be experimentally determined by various methods. The IBPGR Advisory Committee on Seed Storage have advised that, for use in genebanks, the ISTA methods are accurate and acceptable. However, considering that the cost of germplasm is high and moisture content determination is destructive, it has been recommended that the ISTA methods should be modified by reducing the weight of seeds required for each replicate whilst increasing the accuracy of the weighings.

The ISTA has only recommended standard methods for common crop seeds (Table 2). There are therefore no standard methods for many of the species stored in genebanks. The ISTA methods can be applied to seeds of these other species where appropriate as explained in the following pages.

Table 2. Species whose moisture content should be determined by ISTA methods

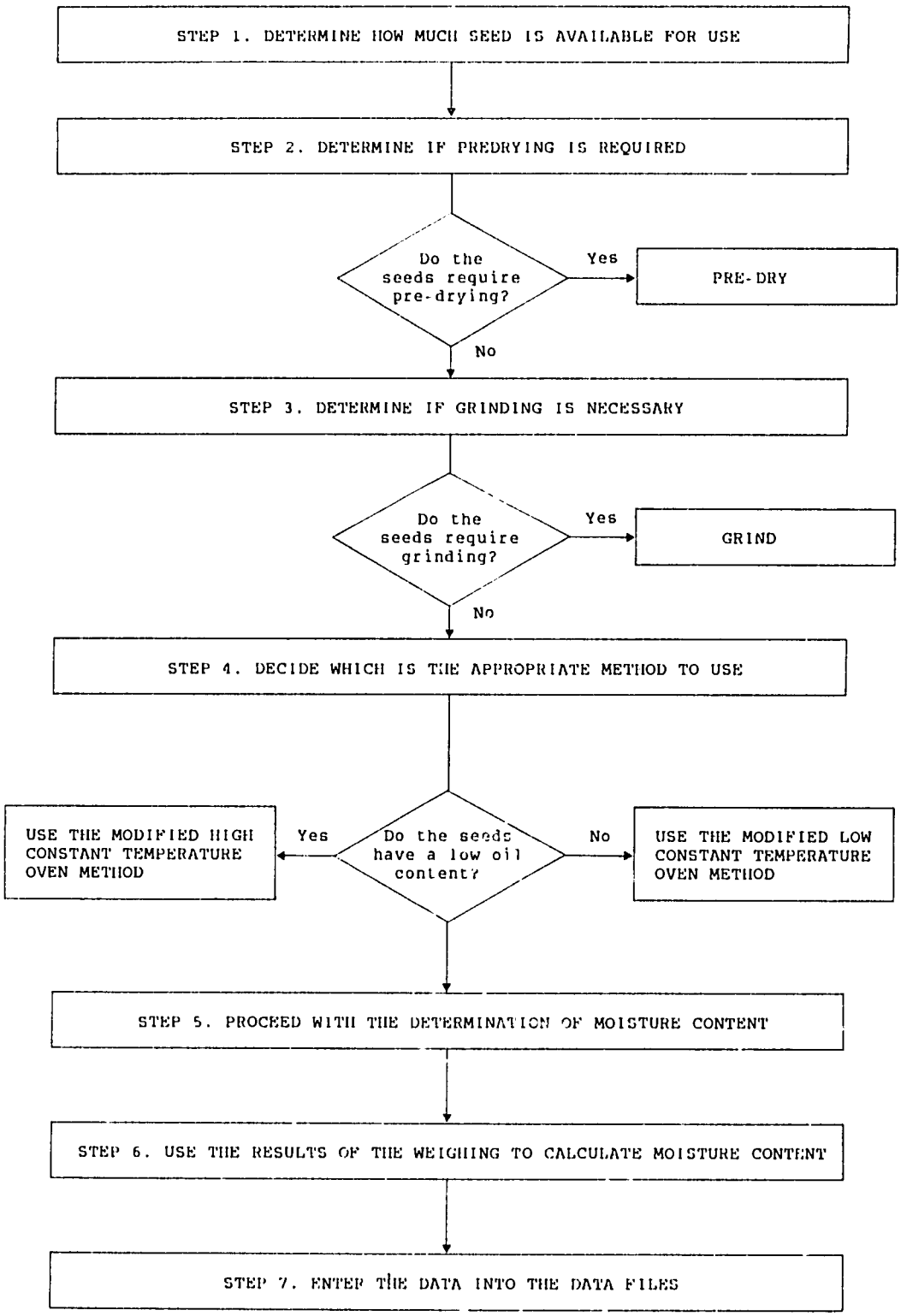
Modified low constant temperature oven method

Brassicas	Falseflax (*Camelina*)	Sesame (*Sesamum*)
Castor (*Ricinus*)*	Flax (*Linum*)	Soyabean (*Glycine*)*
Pepper (*Capsicum*)	Groundnut (*Arachis*)*	All tree species
Cotton (*Gossypium*)*	Onion (*Allium*)	
Eggplant (*Solanum*)	Radish (*Raphanus*)	

Modified high constant temperature oven method

Alfalfa (*Medicago*)	Clover (*Trifolium*)	Rice (*Oryza*)*
Asparagus (*Asparagus*)	Cocksfoot (*Dactylis*)	Rye (*Secale*)*
Barley (*Hordeum*)*	Cress (*Lepidium*)	Ryegrass (*Lolium*)
Bean (*Phaseolus*)*	Crested dogtail (*Cynosurus*)	Sainfoin (*Onobrychis*)
Beet (*Beta*)	Cucumber (*Cucumis*)	Serradella (*Ornithopus*)
Bentgrass (*Agrostis*)	Cumin (*Cuminum*)	Sorghum (*Sorghum*)*
Bermuda grass (*Cynodon*)	Dallisgrass (*Paspalum*)	Squash (*Cucurbita*)
Black salsify (*Scorzonera*)	Fescue (*Festuca*)	Sweetclover (*Melilotus*)
Bluegrass (*Poa*)	Foxtail (*Alopecurus*)	Tall oatgrass (*Arrhenatherum*)
Brome (*Bromus*)	Lettuce (*Lactuca*)	Timothy grass (*Phleum*)
Buckwheat (*Fagopyrum*)*	Lupin (*Lupinus*)*	Tomato (*Lycopersicon*)
Canarygrass (*Phalaris*)	Maize (*Zea*)*	Trefoil (*Lotus*)
Caraway (*Carum*)	Millet (*Panicum*)	Tufted hairgrass (*Deschampsia*)
Carrot (*Daucus*)	Oat (*Avena*)*	Velvetgrass (*Holcus*)
Chervil (*Anthriscus*)	Parsley (*Petroselinum*)	Vetch (*Vicia*)*
Chickory (*Cichorium*)	Pea (*Pisum*)*	Watermelon (*Citrullus*)*
Chickpea (*Cicer*)*	Rhodes grass (*Chloris*)	Wheat (*Triticum*)*

* Indicates grinding is required for moisture content determination


STEP 1. DETERMINE HOW MUCH SEED IS AVAILABLE FOR USE
STEP 2. DETERMINE IF PREDRYING IS REQUIRED
Do the seeds require pre-drying?
Yes
PRE-DRY
No
STEP 3. DETERMINE IF GRINDING IS NECESSARY
Do the seeds require grinding?
Yes
GRIND
No
STEP 4. DECIDE WHICH IS THE APPROPRIATE METHOD TO USE
Do the seeds have a low oil content?
Yes
USE THE MODIFIED HIGH CONSTANT TEMPERATURE OVEN METHOD
No
USE THE MODIFIED LOW CONSTANT TEMPERATURE OVEN METHOD
STEP 5. PROCEED WITH THE DETERMINATION OF MOISTURE CONTENT
STEP 6. USE THE RESULTS OF THE WEIGHING TO CALCULATE MOISTURE CONTENT
STEP 7. ENTER THE DATA INTO THE DATA FILES

How to determine seed moisture content

STEP 1. DETERMINE HOW MANY SEEDS ARE AVAILABLE FOR THE TEST

1. The total weight of seeds used for a moisture content determination should be sufficient to make the test accurate and yet not be wasteful of seeds. The sample should be fully representative of the accession and a minimum of ten seeds should be used to prepare the samples for the test.

2. The ISTA rules for seed testing recommend that two replicates of 4 g of seeds are used for the determination of moisture content for official seed testing (ISTA, 1976b). However, this weight of seeds is not appropriate for use in genebanks because large numbers of small seeds are needed, unnecessarily depleting the accession sample size. Therefore, it is suggested that approximately 0.5 g of seeds per replicate should be used for the moisture content determination of seeds in genebanks.

3. The lower the weight of seed used, the more accuracy is required to achieve a true result. Small samples should be weighed with an analytical balance to four decimal places using light-weight dishes, so that the ratio of the weight of the seeds and the dish is not too disproportionate.

STEP 2. DETERMINE IF PRE-DRYING IS REQUIRED

1. Use your experience to predict moisture content as explained in the previous Section.

2. If the seeds are very moist, pre drying may be necessary. ISTA requires pre-drying for seeds of the species which require grinding listed in Table 2 (see page 30) of more than 17% seed moisture content, for seeds of soyabean of more than 10% moisture content, seeds of rice of more than 13% moisture content and seeds of other species of more than 30% moisture content.

STEP 3. DETERMINE IF GRINDING IS NECESSARY

1. Check the ISTA list of species to see if the seeds require grinding (Table 2).

2. If the seeds are not on the list but are larger than wheat seeds, grinding is required.

STEP 4. DECIDE WHICH IS THE APPROPRIATE METHOD TO USE

1. Two methods are recommended: one for oily seeds and one for all others.

2. If there is a recommended ISTA method for seeds of this species (see Table 2 on page 30), use the modified version of this method.

3. If the species is not included in Table 2, but has oily seeds use the modified ISTA Low Constant Temperature Oven Method.

STEP 5. PROCEED WITH THE DETERMINATION OF MOISTURE CONTENT

The standard ISTA determination methods have been modified for use in genebanks in this manual by decreasing the weight of seeds required for each replicate and the use of balances which weigh to more decimal places.

Notes and Examples

Great care is required especially with small samples when doing the determination to ensure that the results are valid. In order to reduce experimental error, light-weight dishes can be made from strong aluminium foil, but these are fragile to handle and covers are not easy to make.

Always work with care and finish one sample at a time. Do not leave the dishes open in the laboratory between weighings because the seeds will either lose or absorb water from the air and small changes in weights can result in large differences in the calculations when the amount of seed used is small.

Grinding should be done carefully to prevent errors in moisture content determination through water loss or absorption during the process. Hard seeds can be difficult to grind and a pestle and mortar may be useful. Very moist seeds will also be difficult to grind and a sharp knife and chopping board can be used to cut them into small pieces of the recommended sizes.

High temperatures cannot be used to determine the moisture content of oily seeds because the oil will also vaporize and give a false result of water plus oil content. Temperatures of just over 100°C allow evaporation of water and minimal vaporization of oils. If you are in doubt as to which method to use, choose the low temperature method for safety.

Modified ISTA low constant temperature oven method

1. If pre-drying is not required, weigh out approximately the total weight of seeds to be used, as determined in Step 1 on page 32.

2. If pre-drying is required, weigh out accurately two sub-samples of between 1 - 1.5 g of seeds. Pre dry these samples in a warm dry place overnight. Weigh again, writing all the weights in the note book.

3. If grinding is necessary, grind the seeds of each sample, directly before weighing, into pieces not larger than 4 mm for legumes and tree seeds and 0.5 - 1 mm for cereals.

4. Keep the ground seeds in a covered dish and go directly to the weighings.

5. Weigh one clean numbered dish and cover accurately to 4 decimal places using an analytical balance. Write the weight in the notebook.

6. Add approximately 0.5 g of the prepared seeds distributed evenly over the base of the dish, replace the cover and accurately weigh the dish and cover and write this weight in the notebook.

7. Place the dish in a safe place and continue to do the second replicate and additional samples in the same way.

8. When all samples have been weighed into numbered dishes, place each dish on top of its numbered lid in the oven at 101 - 105°C.

9. Wait for the oven to reach this temperature and heat the samples for 15 - 17 hours.

10. Remove the dishes from the oven, replace their covers and place in a desiccator to cool for 30 to 45 minutes at room temperature.

11. Remove the dishes one by one from the desiccator and immediately weigh each dish and cover and note the weight in the notebook. Do not leave the desiccator open during the weighings.

Modified ISTA high constant temperature oven method

1. If pre drying is not required, weigh out approximately the total weight of seeds to be used, as determined in Step 1 on page 32.

2. If pre-drying is required, weigh out accurately two sub-samples of between 1 - 1.5 g of seeds. Pre-dry these samples in a warm dry place overnight. Weigh again, writing all the weights in the note book.

3. If grinding is necessary, grind the seeds of each sample, directly before weighing, into pieces of not larger than 4 mm for legumes and tree seeds and 0.5 - 1 mm for cereals.

4. Keep the ground seeds in a covered dish and go directly to the weighings.

5. Weigh one clean numbered dish and cover accurately to 4 decimal places using an analytical balance. Write the weight in the notebook.

6. Add approximately 0.5 g of the prepared seeds distributed evenly over the base of the dish, replace the cover and accurately weigh the dish and cover and write this weight in the notebook.

Notes and Examples

Use a standard table form to record the results and simplify your work and calculations.

After heating, make sure that the dishes are put directly in the desiccator so that the dry seeds do not absorb more moisture. Do not try to close a desiccator with hot dishes inside until the air has expanded, or the expansion of air can break the seal and cause the lid to slide off. After cooling there will be a slight vacuum in the desiccator and the lid may be difficult to remove. Grease the lid of the desiccator to make it easier to remove. The use of a desiccator with a tap in the lid can prevent these problems.

Equipment

Grinder
Heat resistant dishes with covers
Analytical balance
Forced draught oven
Desiccator
Silica gel
Tongs and oven cloth

7. Place the dish in a safe place and continue to do the second replicate and additional samples in the same way.

8. When all samples have been weighed into numbered dishes, place each dish on top of its numbered lid in the oven at 130 - 133°C.

9. Wait for the oven to reach this temperature and heat the samples for 2 hours for cereals, 4 hours for maize and 1 hour for all other species on the ISTA list.

10. Remove the dishes from the oven, replace their covers and place in a desiccator to cool for 30 to 45 minutes at room temperature.

11. Remove the dishes one by one from the desiccator and immediately weigh each dish and cover and write the weight in the notebook. Do not leave the desiccator open during the weighings.

STEP 6. USE THE RESULTS OF THE WEIGHING TO CALCULATE MOISTURE CONTENT

1. Moisture content is calculated on a wet weight basis and expressed to one decimal place.

2. Replicates should not differ by more than 0.2%.

3. For samples tested without pre-drying use the following formula:

$$\text{\% Moisture Content} = \frac{\text{Weight of fresh seeds} - \text{Weight of dry seeds}}{\text{Weight of fresh seeds}} \times 100\ \%$$

4. For samples which have been pre-dried, use the following formula:

$$\text{Final \% moisture content} = S1 + S2 - \frac{S1 \times S2}{100}\ \%$$

where S1 = Percentage moisture content from first stage of drying
and S2 = Percentage moisture content from second stage of drying

STEP 7. ENTER THE DATA INTO THE DATA FILES

1. If the standard method was used, enter the percentage moisture content of each accession into the data file for that accession.

2. If the standard method was not used, also enter a note of the method used to determine it.

Example 1. One stage drying

Material	Dish number	Wt. of dish (g)	Wt. of dish and fresh seed (g)	Wt. of dish and dry seed (g)
C124 rep. 1	35	10.3245	14.8668	14.6356
C124 rep. 2	29	10.1442	14.9948	14.7485

Calculation:

Rep. 1: % moisture content $= \frac{14.8668 - 14.6356}{14.8668 - 10.3245} \times 100 = 5.1\%$

Rep. 2: % moisture content $= \frac{14.9948 - 14.7485}{14.9948 - 10.1442} \times 100 = 5.1\%$

Example 2. Two Stage Drying (Pre drying)

Material	Dish number	Wt. of dish (g)	Wt. of dish and fresh seed (g)	Wt. of dish and dry seed (g)
First stage of drying (pre-drying)				
Z099 rep. 1	07	20.4112	35.6615	33.2419
Z099 rep. 2	08	21.3121	36.7446	34.2689
Second stage of drying				
Z099 rep. 1	61	10.2323	14.8839	14.6532
Z099 rep. 2	62	10.4132	14.9647	14.7475

Calculation:

Rep. 1 % moisture content from pre drying (S1) $\frac{35.6615 - 33.2419}{35.6615 - 20.4112} \times 100 = 15.9\%$

% moisture content from second drying (S2) $= \frac{14.8839 - 14.6532}{14.9647 - 10.4132} \times 100 = 5.0\%$

Final % moisture content $= 15.9 + 5.0 - \frac{15.9 \times 5}{100} = 20.0\%$

Rep. 2 % moisture content from pre drying (S1) $= \frac{36.7446 - 34.2689}{36.7446 - 21.3121} \times 100 = 16.0\%$

% moisture content from second drying (S2) $= \frac{14.9647 - 14.7475}{14.9647 \quad 10.4132} \times 100 = 4.8\%$

Final % moisture content $= 16.0 + 4.8 - \frac{16.0 \times 4.8}{100} = 20.0\%$

Table of moisture content determination methods for seeds stored in your genebank

Fill in this table to use as your future guide:

Species	Pre drying	Grinding	Oven temperature	Drying period

SECTION IV

Seed Drying

What is meant by seed drying?

This is the reduction of seed moisture content to the recommendod levels for seed storage, using techniques which will not be detrimental to seed viability.

Why are seeds dried?

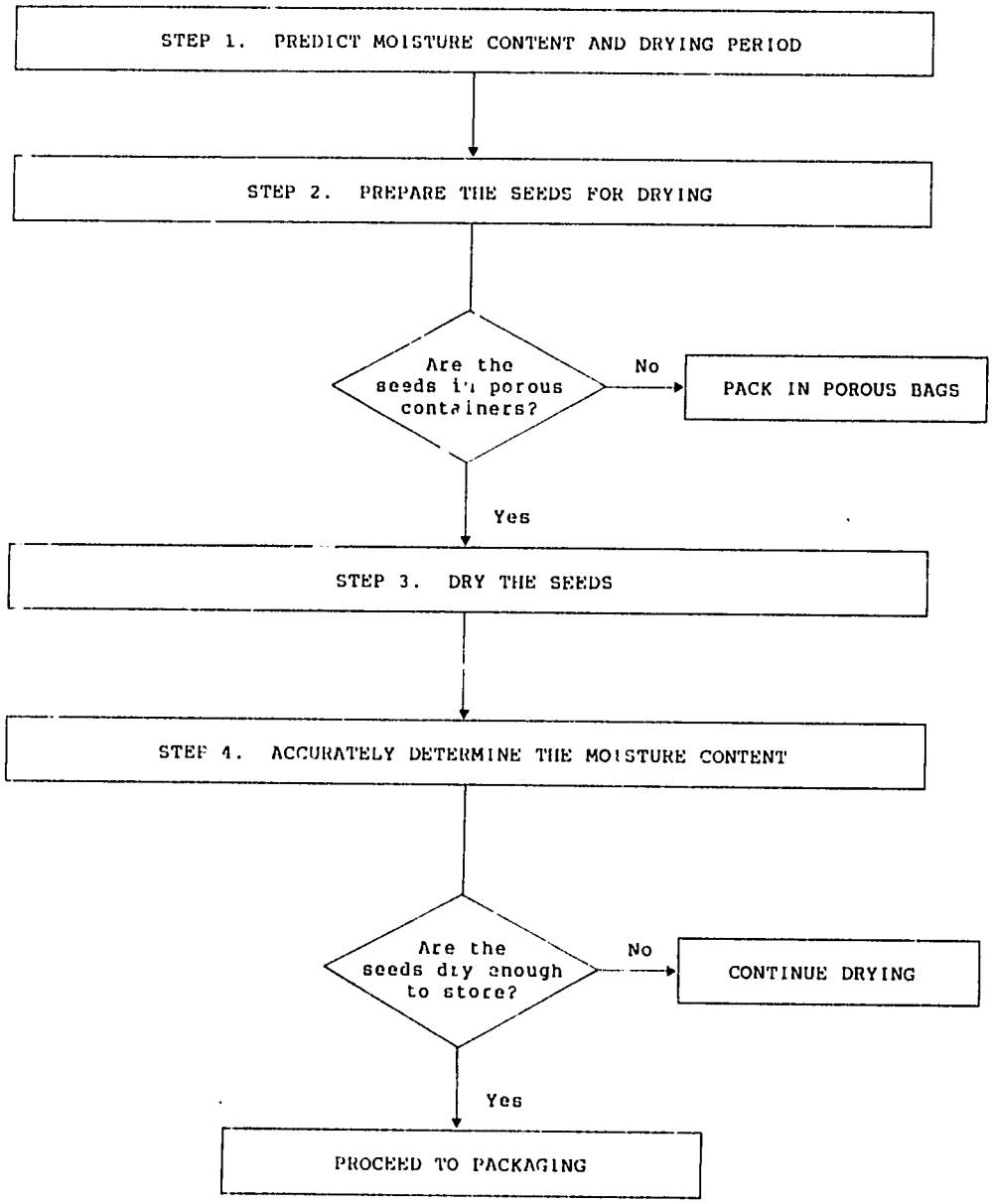
Seeds which are dry will retain their viability for longer periods of storage in genebanks. It is recommended that, in general, seeds should be dried to between 3 - 7% moisture content for long-term storage, except in certain cases where it has been shown that low moisture content causes problems e.g. soyabean should be dried to about 8% moisture content. Drying has an independent effect from temperature on viability during storage and adequate drying could prolong viability for reasonably long periods without cold storage.

When should seeds be dried?

The drying process should begin as soon as possible after the receipt of the seeds to avoid unnecessary deterioration. It can take up to several weeks to lower the moisture content to the low levels required for good storage.

How should seeds be dried?

Several methods are available for drying seeds. Some are more suitable in certain environments and safer for the viability of the seeds than others. Drying seeds in an atmosphere of reduced relative humidity is recommended. The lower the humidity the faster the seeds will dry and the lower their final moisture content. A relative humidity of 10 - 15% and a temperature of 15°C are recommended by the IBPGR Advisory Committee on Seed Storage: try to obtain these conditions as closely as possible. The following methods can be used with safety and one should be chosen depending on the equipment available.


STEP 1. PREDICT MOISTURE CONTENT AND DRYING PERIOD
STEP 2. PREPARE THE SEEDS FOR DRYING
Are the seeds in porous containers?
No
PACK IN POROUS BAGS
Yes
STEP 3. DRY THE SEEDS
STEP 4. ACCURATELY DETERMINE THE MOISTURE CONTENT
Are the seeds dry enough to store?
No
CONTINUE DRYING
Yes
PROCEED TO PACKAGING

Prediction of drying period

If there is no previous experience of drying seeds of a particular species it may be necessary for you to do some experimental work to predict the approximate drying period. Seeds dry at an exponential rate until the equilibrium moisture content is reached. The rate of drying of different seed lots of the same species is fairly constant for seeds dried under the same environmental conditions. The length of the drying period can be predicted in one of two ways:

Prediction of the correct drying period by weight loss

1. Predict the current percentage moisture content and the percentage moisture content required for storage.

2. Weigh the seed sample.

3. Use these three values to calculate the weight of seeds at the required moisture content by using the following formula:

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

4. Weigh the seed sample at regular intervals during the drying period until the weight of the seeds has reached this calculated value.

Prediction of drying period from mean drying curves

1. Do not waste valuable seeds for this, but use either excess seeds or those which are being discarded because they have lost viability.

2. Take at least two lots of seeds of the species and place to dry, using the method that you will use in practice.

3. Remove a sample of seeds and do an accurate determination of moisture content for each seed lot as described in Section III (see page 32).

4. The mean of the two tests can be used as a guide because other seed lots of the same species should dry at a similar rate.

5. Repeat the determination daily and plot a graph of the drying curve (mean percentage moisture content against time) for that species under these drying conditions. Your graph will be similar to the one on page 44.

6. The work can be repeated with seeds of all species which are of interest and their drying curves plotted for different conditions.

Notes and Examples

Equipment

The prediction of the drying period will be more accurate if the initial moisture content is determined. However, this is a waste of seeds when moisture content can be adequately predicted.

Coarse balance

1000 g of seeds with 12% moisture content were dried to 5% moisture content. What would be the weight of these seeds after drying?

Substitute the values in the equation on the facing page:

$$\text{Final seed weight} = 1000 \times \frac{(100 - 12)}{(100 - 5)} \text{ g}$$

$$= 926.3\text{g}$$

Therefore when the initial 1000g of seeds have been dried to 926.3g, their moisture content will have decreased from 12% to 5%.

Grinder
Heat resistant dishes with covers
Analytical balance
Forced draught oven
Desiccator
Silica gel
Tongs and oven cloth

If the seeds are very small and are dried quickly, the moisture content can be determined more frequently during the drying period to plot a more accurate graph.

How to use the drying curves

1. Use the graph that you have prepared for seeds of a particular species being dried under those conditions.

2. Predict the current percentage moisture content of the accession by using the table of equilibrium moisture contents on pages 26 and 27. Select the final percentage moisture content that is required for storage of this species.

3. Find the value for each of the percentage moisture contents on the vertical 'Y' axis, follow this across to the curve and read off the day on the horizontal 'X' axis for each of the moisture contents. The difference between these two values is the drying period in days.

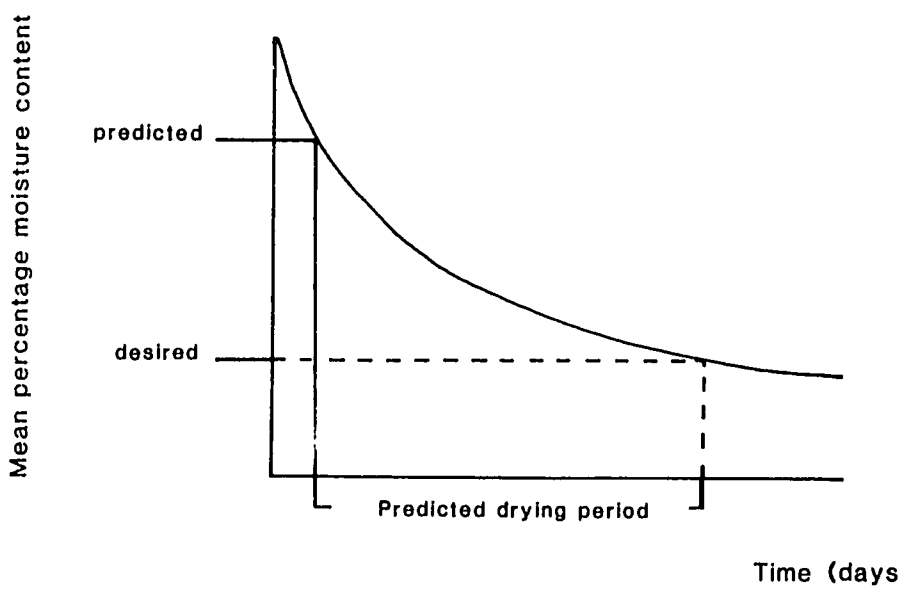


Figure 2. Typical drying curve

Notes and Examples

Example of how to predict the drying period by using a typical drying curve for small seeds of crops such as onion or cabbage, dried quickly under low relative humidity conditions:

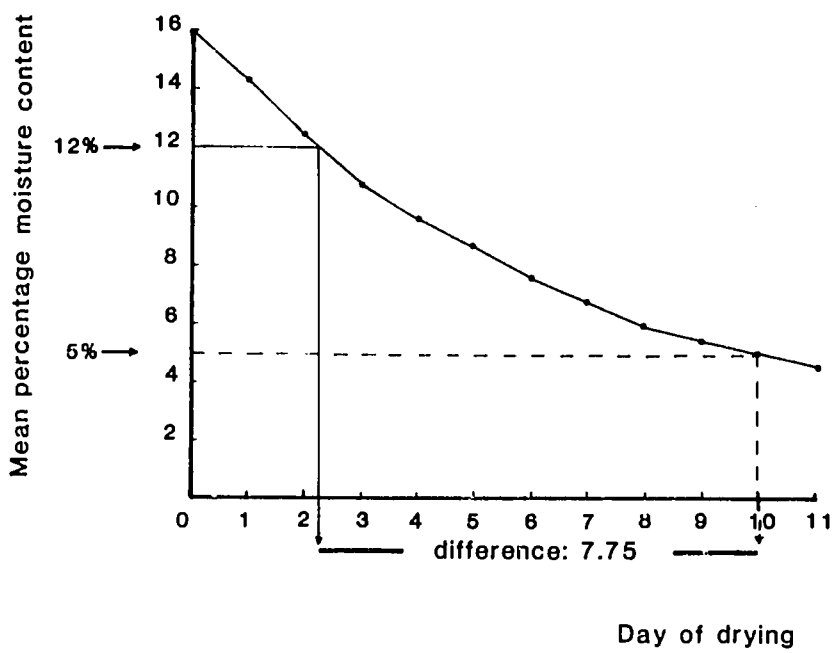


Figure 3. Typical drying curve of small seeds

Predict the moisture content by using the table of equilibrium moisture contents for your genebank which you have prepared (page 27). As an example take this as 12% and the desired moisture content after drying as 5%. Using the graph, the intersects from the curve to the time axis show the values 10 and 2.25 days.

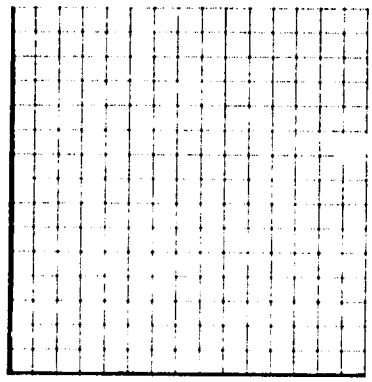
Therefore, the time to dry the seeds to 5% moisture content under the same conditions will be the difference between 10 and 2.25 days. This can be approximated to the nearest day, in this case 8 days.

In practice it is unlikely that using the graph will give you a whole number of days, therefore you should approximate to the nearest day.

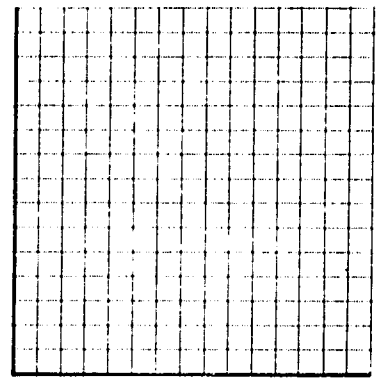
Graphs of mean drying curves in your genebank

Plot graphs in the space below to use for future reference:

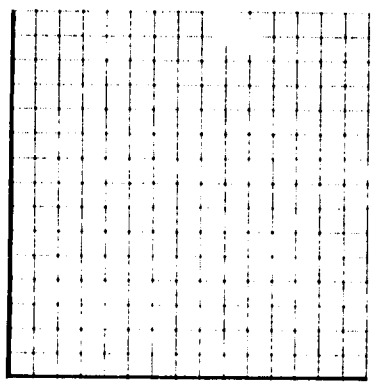
1. Species:
   Temperature:
   Relative humidity:



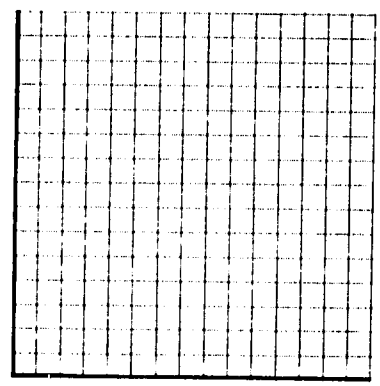
2. Species:
   Temperature:
   Relative humidity:



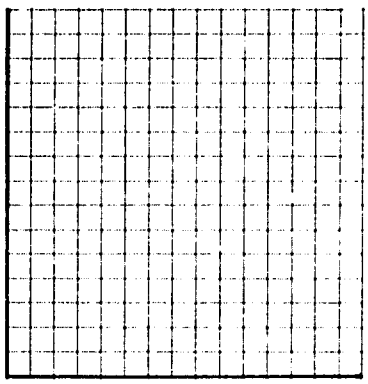
3. Species:
   Temperature:
   Relative humidity:



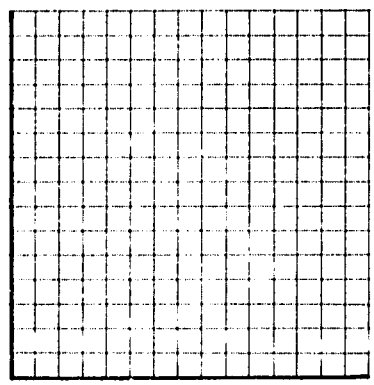
4. Species:
   Temperature:
   Relative humidity:



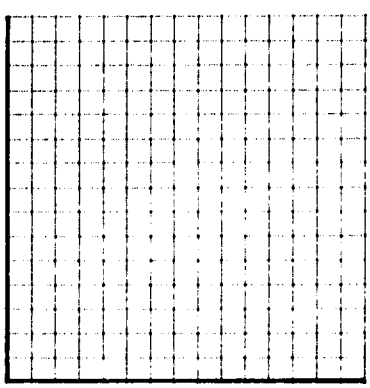
5. Species:
   Temperature:
   Relative humidity:



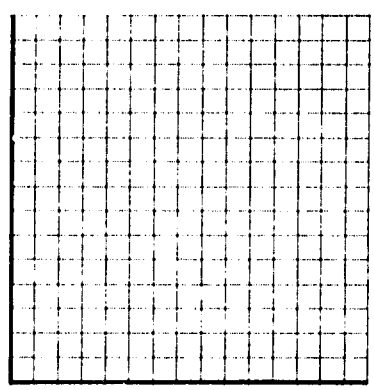
6. Species:
   Temperature:
   Relative humidity:



7. Species:
   Temperature:
   Relative humidity:



8. Species:
   Temperature:
   Relative humidity:



Seed drying procedures

STEP 1. PREDICT MOISTURE CONTENT AND DRYING PERIOD

1. Use the experience and techniques already described in Section III to predict the moisture content of the accessions.

2. Predict the length of drying period required from your previous experience of drying seeds of the same species with similar moisture contents, as already described. Information on drying periods can also be found in Cromarty, Ellis and Roberts (1982).

STEP 2. PREPARE THE SEEDS FOR DRYING

1. Place each accession in labelled paper envelopes or cloth bags.

2. Do not put too many seeds in the same bag. One accession can be split into several labelled bags to help rapid drying.

STEP 3 DRY THE SEEDS

Several methods are available for drying seeds. The safest methods rely on leaving the seeds in an environment of low relative humidity and allowing the seed moisture content to reach equilibrium with this at relatively low temperatures. Seeds will equilibrate with the relative humidity at different rates, depending on species, seed size and conditions. Seeds dry quicker when first placed in the room and more slowly as the low moisture contents suitable for storage are approached. Two common and safe methods are described here:

Use of a dehumidifier dryer

1. Use a dehumidifier dryer to dry a small volume of air in a drying room or other limited space. The humidity of this room will depend on its size, the ambient humidity of the area and the efficiency of the dehumidifier.

2. Place the seeds which have been packaged in labelled porous containers in the drying area.

3. 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.

4. Measure the relative humidity and maximum and minimum temperatures of the room daily.

5. Leave the seeds in the drying area until the moisture content is predicted to be in the range required for storage.

Use of silica gel

1. Use deep blue silica gel in an enclosed space, such as a desiccator, can or glass jar with an air-tight seal.

2. Place either the silica gel or the seeds in a porous bag.

3. Use a weight of silica gel equal to the weight of seeds for rapid drying.

Notes and Examples	Equipment
Predict the moisture content of each accession and the number of days to reach the required moisture content. Remember that seeds will dry more slowly as the moisture content decreases towards the equilibrium moisture content. At low moisture contents a difference of one or two days of drying will not have a large effect on seed moisture content.	
Make sure that the bags are porous. Seeds will not dry in plastic bags!	
Choose the best drying method for your own genebank. In tropical countries with high relative humidities, it will be more difficult to hold a drying room at very low relative humidity. A combination of methods is possible. Silica gel can be used after the first drying period if the relative humidity is too high to allow the seeds to equilibrate to low moisture contents. Do not use sun drying because it is believed to affect long term seed viability of some species. Heated air drying should also be avoided for the same reason. Aim for 10 - 15% relative humidity and 15°C as the optimum drying conditions.	Dehumidifier dryer or cabinet Envelopes Porous boxes or containers Maximum minimum thermometer Aspirated hygrometer Racks Coarse balance Spatula/spoons
Desiccators are useful but expensive. Cans and jars with air-tight seals are cheaper and adequate and also allow separation of seeds of different accessions. Silica gel is cheap and easy to use. It can be made into permanent packages of fixed weight in fine cloth bags. These can be heated in the oven without damage and are quicker and easier to handle than loose silica gel.	Silica gel Desiccators, air-tight cans or bottles Forced draught oven from 50 - 200°C Coarse balance Spatula/spoons

4. Place the required weight of silica gel in a desiccator, jar or can with the correct weight of seeds. When using cans or jars make sure that the silica gel is in close proximity to, but not touching, the seeds.

5. Place the containers in a room held at approximately 15°C whilst the seeds are drying.

6. Change the silica gel daily or when the colour changes from deep blue to pale blue or pink.

7. Heat the pale blue or pink silica gel in an oven above 100°C until it turns deep blue again, when it is ready for re use. Store in an air-tight container.

8. Leave the seeds with fresh changes of silica gel in the container until the moisture content is predicted to be in the range required for storage.

STEP 4. ACCURATELY DETERMINE THE MOISTURE CONTENT

1. When you consider, from experience, that the seeds are dry enough, remove a sub-sample from each accession and carry out a moisture content determination as explained in Section III (see page 32).

2. When the moisture content is between 3 - 7% for most species, go to packaging (Section VI on page 70). Low moisture contents are detrimental to the viability of the seeds of some crops. These seeds should be treated with care and not dried to low moisture levels, e.g. soyabean should not be dried below 8% moisture content.

3. If the moisture content is not low enough, continue to dry for a further period as described above. When the moisture content is predicted to be in the correct range for seed storage, carry out an experimental determination as described in Section III on page 32.

Notes and Examples	Equipment
The silica gel is used to reduce the relative humidity of the atmosphere surrounding tho seeds. The seeds then dry by equilibration with their surroundings.	
At this point it is necessary to do an experimental determination of moisture content to ensure that predictions of storage life of this accession are as accurate as possible. Make sure that seeds which have been dried are kept in the drying area or moisture proof containers whilst tests are carried out to prevent absorption of moisture from the surroundings.	Grinder Heat resistant dishes with covers Analytical balance Forced draught oven Desiccator Silica gel Tongs and oven cloth

Table of approximate drying times in your conditions

Fill in this table for your future reference:

Species	Mean initial percent moisture content	Days required to reach 5% moisture content
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SECTION V

Seed Viability

What is seed viability?

The viability of the seed accession is a measure of how many seeds are alive and could develop into plants which will reproduce themselves, given the appropriate conditions.

Why do we test seed viability?

It is important to know that the seeds that are stored in a genebank will grow to produce plants. Therefore they must have a high viability at the start and during storage. The viability of seeds at the start of storage will also determine, within the environmental conditions, the storage life of the accession.

When should viability be determined?

Viability will need to be determined at the start of storage and at regular intervals during storage to predict the correct time for regeneration of the accession. The viability test takes from a few days to weeks or even months to give an accurate result. If possible the results should be available before the seeds are packaged and placed in the genebank so that poor quality seeds can be identified and regenerated before storage. Where the viability cannot be determined before storage, the seeds should be placed into long-term storage to ensure their safety whilst awaiting the results of the test.

How should viability be determined?

The most accurate test of viability is the germination test and this will be described here. The germination test is made under controlled conditions to find out how many seeds will germinate and produce normal seedlings which could develop into normal reproductively mature plants. The IBPGR Advisory Committee on Seed Storage recommends that for the initial germination test of species where a reasonable germination technique is available, a minimum of two replicates using 200 seeds (100 seeds per replicate) is acceptable, providing that germination is above 90%. If not, a further 200 seeds should be tested as before and he overall result for seed viability taken as the mean of the two tests. Other biochemical tests are available to test viability. These have the advantage of being quicker, but are not as accurate and require considerable skill and practice in their implementation and interpretation. These are not recommended by the IBPGR Advisory Committee on Seed Storage for general use as tests for seed viability.

Germination testing

How is germination tested?

There are many methods available which can be used to test germination. Seeds of different species have different requirements for light, substrate, temperature and water during germination. For an initial germination test the IBPGR Advisory Committee on Seed Storage recommends that a fixed sample size test should be used.

How do we choose which conditions to use in the germination test?

There is no general set of conditions which can be used to germinate seeds of all different species. Each species is different and must be treated separately. Guidelines for germination of seeds of the most common crop species and some of their wild relatives which are likely to be stored in genebanks are given in Vol II of Ellis, Hong and Roberts (1985). Seeds of some species are more tolerant and can germinate under a wide range of conditions but complete germination will only be achieved under optimum conditions.

Why do some seeds fail to germinate in the test?

Two main reasons for the failure of seeds to germinate in suitable conditions are because they are either dead or dormant. Dead seeds can be identified because they usually soften and rot during the test as a result of attack by bacteria and fungi. Seeds which remain hard or absorb water, but remain firm and in good condition during the germination test are probably dormant. Seed dormancy is common in some crops straight after harvest (post harvest dormancy) and in many wild species related to crop plants.

How can dormant seeds be stimulated to germinate?

Special treatments are required to overcome seed dormancy. Treatments vary among species and more detailed advice can be found in Vol II of Ellis, Hong and Roberts (1985).

How should non-dormant seeds be germinated?

The most appropriate test conditions should be selected for the test. A summary of suitable test conditions is given in Vol II of Ellis, Hong and Roberts (1985). A fixed sample size germination test should be used as explained here.

STEP 1. DETERMINE THE GERMINATION TEST CONDITIONS

1. Use the normal germination test procedure for the species in your genebank.

2. If there is no standard, ask the curator for advice on the best germination test conditions to use.

3. Check that the equipment and environments are available to fulfil these conditions.

4. If not, a compromise will have to be found using the best possible alternatives available.

5. A fixed sample size germination test using 200 seeds is recommended to determine viability at the start of storage.

STEP 2. PREPARE THE SEEDS FOR THE TEST

1. Take a random sample of seeds from the accession.

2. Count out 200 seeds for each test.

3. Divide these seeds into at least two replicates. More replicates of fewer seeds in each can be used to fit into the equipment at your genebank.

4. In order to prevent damage to the seeds from the rapid uptake of water (imbibition damage), seeds with a low moisture content must imbibe water slowly. Any seeds known to be prone to imbibition damage should be placed in a humid atmosphere to equilibrate as a pre-treatment. This is especially important for many species of legumes.

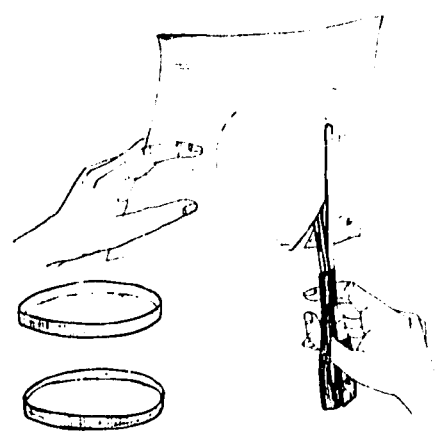
STEP 3. SET UP THE GERMINATION TEST

Although several methods are available for germination testing, it is suggested that you use one of the two major methods described here because these are adaptable to most environments and in general give uniform results.

Germination using absorbent paper

Top of paper method

1. Cut the paper to the size and shape of the dishes.

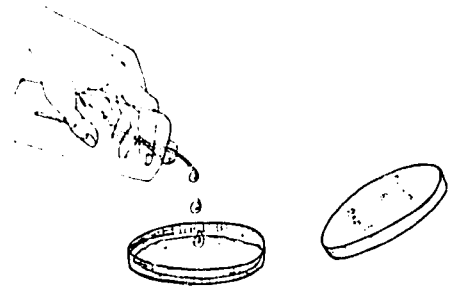


Notes and Examples	Equipment
Choosing the best conditions for germination is difficult because species and even accessions of the same species vary in their requirements. Methods for germinating seeds of major crop species are usually well-documented. Often there is no information on the methods needed for lesser known crops or wild and weed species.	
The viability at the start of storage should be determined accurately to allow more accurate predictions of the storage life of the seeds. The germination test should be statistically accurate and therefore sufficient seeds should be used for the test. The sequential test described in Section VIII (page 86) uses less seeds and is recommended for monitoring but not for the initial test for viability.	
Several types of absorbent paper are available. Choose paper that is relatively cheap, easily available and strong enough to withstand handling and the weight of the seeds when damp. It must be free from chemical residues that could interfere with the germination of the seeds. The most common papers used are towelling, filter paper or rice straw paper. Filter paper and paper towelling are more expensive. Rice straw paper is cheap but fungal spores from the plant material can cause contamination unless heat-treated before use.	
The petri dish/container method has the advantage that the seeds can be observed through the transparent lid and the germinating seeds are therefore easy to count. There is the disadvantage that the dishes soon dry out and need daily watering.	Petri dishes or other containers Filter paper, paper towelling or rice straw paper Water Forceps Incubators Permanent markers

2. Place a layer of paper in each dish. If the paper is too thin use a double layer.

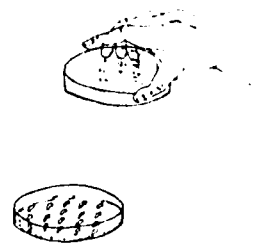
3. Label the top and bottom of each dish with the accession number, number of the replicate and date of the test.

4. Moisten the paper with water.



5. Arrange the seeds in a regular equidistant pattern on the surface of the paper.

6. Add more water if required and replace lid.

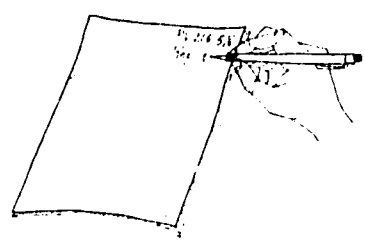


Between paper method

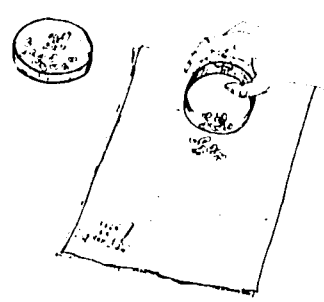
1. Cut the paper to a convenient size to hold one replicate of the seeds when spaced at regular intervals. If the paper is too thin use a double layer.

2. Label each sheet on the outside of the paper at one end with the accession number, replicate of the test and the date of the start of the test.

3. Moisten the paper with water.

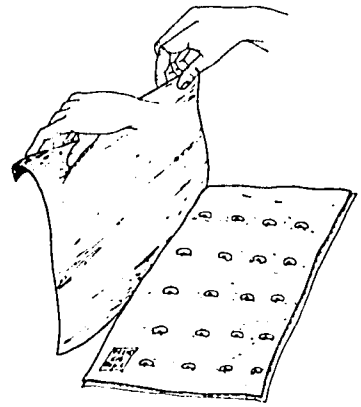


4. Arrange the seeds at regular intervals on the paper, leaving at least two centimetres clear from the edges all round.

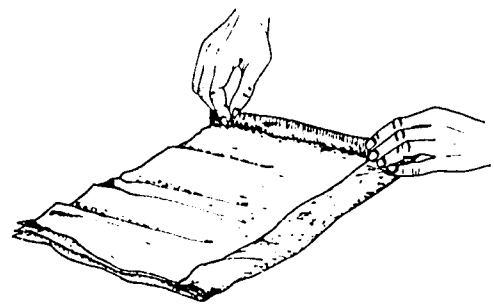


Notes and Examples	Equipment
The thickness of paper used should be more than 2mm when moist.	
The water used must be reasonably free from acid, alkali, organic material or other impurities. It can be either tap, distilled or de-ionized water.	
Seeds arranged in an orderly way are easier to count.	
The between absorbent paper method is cheap and easy to prepare, but the seeds cannot be observed without unrolling the paper. The use of new paper at each test has the advantage that fungal contamination cannot be carried from one test to another.	Filter paper, paper towelling or rice straw paper Plastic bags Water Forceps Incubators Permanent marker
Leave enough room around the edges of the paper so that the paper can be folded back without damaging the seeds.	

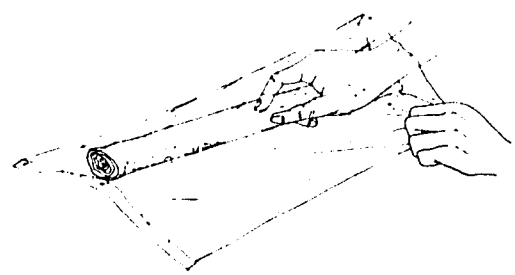
5. Cover the seeds with another sheet of paper and fold in the edges to prevent the seeds from falling out.



6. Roll the paper loosely towards the end with the label.

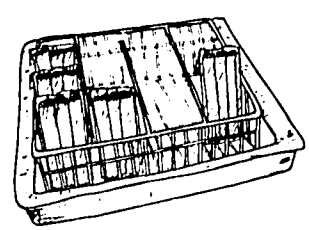


7. Place the rolled papers inside ventilated plastic bags or boxes.



8. Put these in an upright position in wire baskets or plastic boxes.

9. Keep the paper moist with water.



Notes and Examples

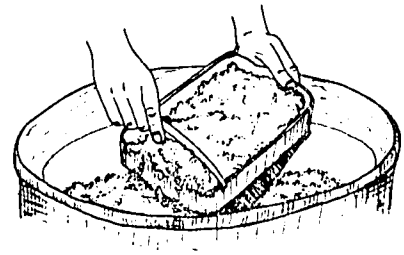
Folding the edges is especially important for large spherical seeds which tend to fall out because of their weight and shape.

Do not roll the paper too tightly because the compression and lack of oxygen resulting from this can cause the seedlings to develop contorted roots and shoots. These are hard to separate from abnormalities caused by genetic factors.

Oxygen is essential for respiration during germination. Therefore, any containers used should be adequately ventilated.

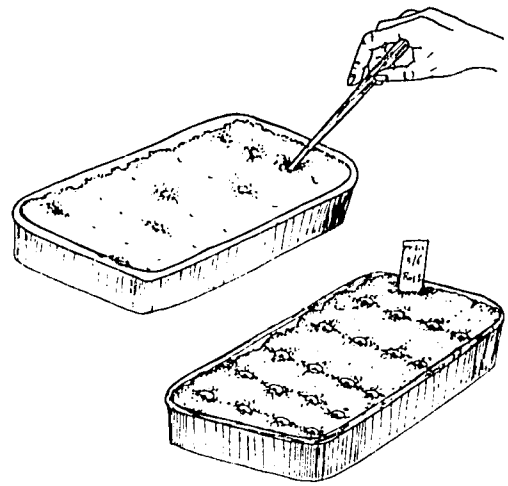
Root and shoot development is geotropic. Placing the rolled papers in an upright position allows seedling development in the natural vertical position.

Germination in sand



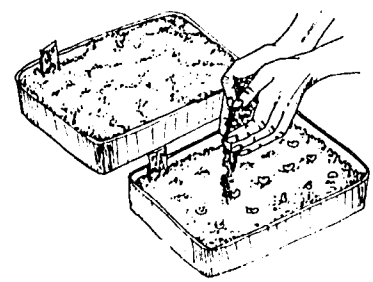
1. Pack clean sterile sand into pots or trays with drainage holes in the bottom.

2. Water the sand until it is moist. Do not use excess water.



3. Make holes in a regular equidistant pattern at about the same depth as the size of the seeds for each replicate of the seeds. Ideally, the distance between holes should be at least three to five times the seed diameter.

4. Prepare a label with the accession number, date of sowing and replicate of the test and place in each pot or tray.



5. Fill seeds from each replicate into the holes and cover with sand.

6. Water the sand again to cover the seeds, but do not make it too wet.

Notes and Examples	Equipment
Fine sand should be used. Make sure that the sand is clean by sterilizing before use. Quarry or river sand is better than shore sand, which must be washed thoroughly to remove all salt.	Sand Labels Permanent markers Pots or trays Tools for planting Water Forceps Incubators
Germination in sand is especially useful for large seeds which are too large to be germinated in petri dishes or too heavy for the between paper method.	
Sprinkle with water slowly, so that the seeds do not float out from the holes and become mixed. Bottom watering is better than top watering.	

STEP 4. CARRY OUT THE GERMINATION TEST

1. Place the prepared germination tests under the light and temperature conditions which were determined in Step 1 on page 56.

2. Keep the substrate moist during the test with water, but do not over-water.

3. Run the test for a sufficient period to determine whether the seeds have germinated, or are dead or dormant. A fixed interval can be used based on previous experience, but the time taken to germinate will vary among species and accessions of the same species.

STEP 5. COUNT THE GERMINATED SEEDLINGS

Germination using absorbent paper

1. Seeds sown on top of paper can be counted either through the transparent cover or after removing the cover of the container. For those between paper, unroll the paper carefully to avoid tearing the paper or damaging the roots of the young seedlings.

2. Count and record the number of normal seedlings in each replicate.

3. Count and record the number of abnormal seedlings in each replicate. Examples of the types of abnormal seedlings found in different species have been described by ISTA (1976b).

4. Once a seed has been counted as germinated, the resulting seedling can be discarded.

5. If the seeds are dormant, as described on page 55, treat with the appropriate technique to stimulate germination as advised in Vol II of Ellis, Hong and Roberts (1985) and continue the test to allow those to germinate.

Germination in sand

1. In this case the number of seedlings emerged from the sand is usually counted because early root growth cannot be seen without disrupting the test.

2. Count and record the number of normal seedlings emerged in each replicate.

3. Count and record the number of abnormal seedlings emerged in each replicate.

4. If any fungal or insect infestation is seen during the germination test, sterilize the sand before using again.

STEP 6. INTERPRET THE RESULTS

1. For each accession, consider the total number germinated in each replicate.

2. Check that the results of the different replicates are compatible by using tolerance tables (see chapter 14 Ellis, Hong and Roberts, 1985).

3. If the results are compatible, calculate the mean percentage viability of that accession from the results of all replicates.

Notes and Examples

Seeds absorb most water during a rapid imbibition phase at the start of the test. Make sure that there is enough water at this stage but remember that too much water at a later stage can limit the oxygen for the seeds and also lead to rotting.

Normal seedlings are those which show the capacity for continued development into normal plants if grown on a suitable substrate and under favourable conditions of water supply, temperature, light and oxygen. Abnormal seedlings are those which do not appear as if they will develop into normal plants. There are three classes of abnormality - damage, deformity and decay.

Tolerance tables can be consulted to check that the results of your work are valid because all replicates of a test should be compatible and within certain limits.

4. If the results are not compatible consult the curator for advice on how to proceed.

STEP 7. DECIDE WHETHER TO REPEAT THE TEST

1. Consider the mean percentage viability of the accession.

2. If it is above 90%, accept the test as valid and use this value as the true viability.

3. If the result is 90% or below, repeat the test using a further 200 seeds following the same procedures.

4. Calculate the mean percentage viability from the results of the two tests and use this as the overall test result.

STEP 8. ENTER THE DATA INTO THE FILES

1. If the standard test for that species in your genebank has been followed exactly, enter the percentage viability into the inventory data file for each accession.

2. If a standard test has not been used, either enter a reference to the full germination data or state the conditions which vary from the standard test.

Notes and Examples

Here a problem may arise. If the results of one replicate are not compatible with the others, the curator will have to decide either to repeat the test or accept these results. It may be possible to accept the results of other replicates and ignore the one that is not compatible only if there is a good reason why one replicate differs, e.g. the dish dried out during the test, etc.

The conditions of the germination test can influence the number of seeds which germinate, especially if seed vigour is low. Optimum conditions allow optimum germination. Therefore, it is important to note the conditions of the test and any deviations from the standard test.

Table of standard germination test regimes for your genebank

Fill in this table for your future reference:

Species	Substrate	Method	Temperature	Light	Test Length	Notes

SECTION VI

Seed Packaging

What is meant by seed packaging?

This is the placing of a counted or weighed sample of seeds of an accession into a container which is then hermetically sealed ready for storage.

Why are seeds packaged?

Seeds are packaged to prevent absorption of water from the atmosphere after drying, to keep each accession separate and prevent contamination of the seeds from insects and diseases.

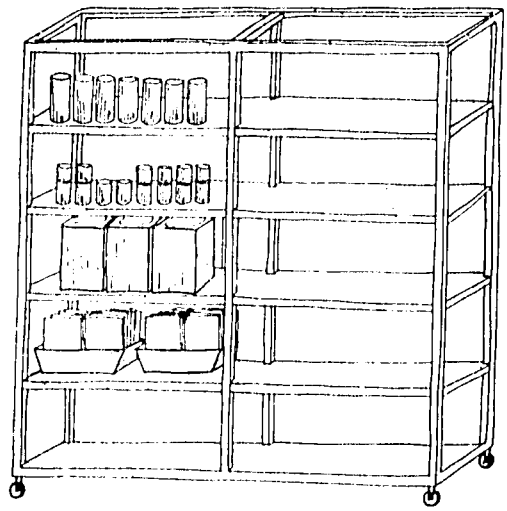
When should seeds be packaged?

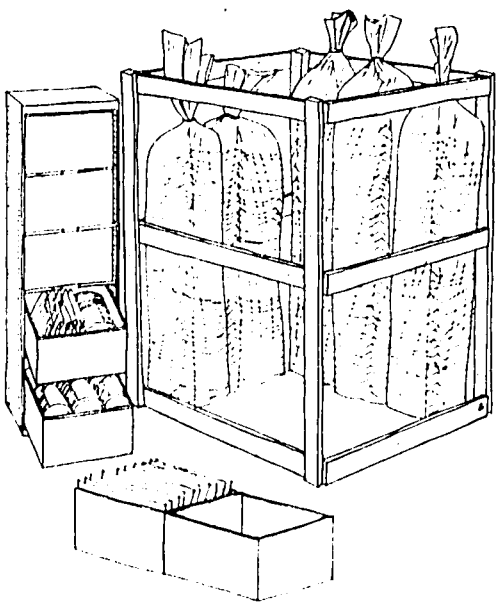
The best time to package seeds is directly after the moisture content has been determined and found to be within the required limits for safe storage. Seeds will always show an equilibrium between their moisture content and the relative humidity of the environment and therefore, if possible, seeds should be packaged into containers and hermetically sealed in the drying room or without delay on being removed from it.

How should seeds be packaged?

Different containers and special equipment for sealing are available for the storage of seeds. Storage containers for base collections should be hermetically sealed and moisture-proof. Cans, bottles, and laminated aluminium foil containers are all acceptable for both base and active collections. The techniques used will vary with the type of container and the equipment that your genebank is using. The general steps outlined in this section could be followed.

Moveable racks make the best use of available space and are ideal to store containers in walk-in stores. Small containers or aluminium foil packets can be filed in boxes for ease of locating indivldual accessions. Coding systems by number or colour are also helpful in exactly locating accessions.





Chest freezers are adequate for the maintenance of small collections. Filing systems have been developed to hold the containers in freezers and allow easy location of material. Some examples of filing systems are shown here.

STEP 1. PREPARE FOR PACKAGING

1. Work in the drying room or, if not possible, expose the seeds to the ambient relative humidity for the shortest possible time.

2. Write on the outside of each container or on an adhesive waterproof label. Also prepare a label for inclusion with the seeds. Record the accession number, date of storage, genus and species if required. Use permanent markers for this.

STEP 2. PACKAGE THE SEEDS

1. Weigh out or count samples of seeds to fit the containers used in your genebank.

2. Fill the labelled containers with the seeds. Add the label prepared for the inside.

3. Seal immediately, so that the moisture content of the seeds does not increase due to equilibration with ambient relative humidity.

4. Note the weight or number of seeds in each container.

STEP 3. ENTER THE DATA INTO THE DATA FILES

1. Enter the relevant data about each accession into the data file.

2. The data should include number of containers per accession, number or weight of seeds per container, type of container (if not standard) and the date of packaging.

STEP 4. CHECK THE QUALITY OF THE CONTAINERS

1. After sealing, make a visual examination of each container to make sure that there is no obvious damage and that the seals do not leak.

2. Any containers that are below standard should be replaced immediately.

3. At regular intervals the containers should be checked to see that they remain in good condition. It is suggested that this check should be carried out routinely once a year and that individual containers should also be checked whenever they are removed from the genebank.

4. If containers are found to have been leaking and the relative humidity of the store was not controlled, determine the seed moisture content by using one of the methods described in Section III (page 32).

5. If the moisture content has risen, dry the seeds back to the required moisture content level as described in Section IV (page 48).

6. Enter the value of the new moisture content into the data files. Make a note that the seeds in that container have been held at increased moisture content for a limited period and dried again.

7. Check the inventory data file for the descriptor 'date of packaging' and make a list of any other accessions which were packaged in similar containers on the same day or one day before or after.

8. Check the containers on this list for leaks and poor condition and replace any that are faulty using the methods described above.

9. Remember that any containers removed from the cold store should be allowed to warm to room temperature before opening. This may take several hours especially with large volumes of seeds.

Notes and Examples

Adhesive labels can be used for the outside of containers, but they must be waterproof and remain adhesive for long periods at low temperatures.

It is important that the containers used should be moisture-proof and sealed. The exact sealing technique will depend on the type of containers and sealing methods that are available.

Laminated aluminium foil bags are easy to package, can be cut to size to save space and can be sealed again after use. However, they are difficult to stack and must be made of good quality material and have good seals or leakage may occur.

Cans are rigid and easy to stack and usually will not break open if dropped. However, some types of cans are not resealable and therefore are expensive to use. A standard size can with only a few seeds inside wastes space in the store.

Glass bottles can usually be sealed again and the amount of seed left is visible. However, a standard size will waste space and bottles are easily broken.

Seeds can be packaged in bulk into large containers and/or sub-samples can be packaged separately into smaller containers. Although the initial packaging period is longer, sub-samples can then be removed quickly without having to remove the bulk of the accession from the store.

If any defective containers are found, it may indicate that containers made or sealed at the same time are also faulty or it may just be one faulty container or seal. Checking those packaged at the same time will show if the problem is widespread.

Equipment

Labels
Permanent markers
Laminated aluminium foil containers, cans or bottles
Machines for sealing
Coarse balance
Scoop/spoons

Summary of seed packaging in your genebank

Fill in this table for your future reference:

Species	Type of container	Optimum number or weight of seeds/container	Sealing method
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SECTION VII

Seed Storage

What is meant by seed storage?

In a genebank, seed storage is the preservation of seeds under controlled environmental conditions which will prolong the viability of the seeds for long periods. Two types of seed stores are used for collections of genetic resources, those holding base collections and those holding active collections. The temperature, relative humidities, seed moisture contents, containers and distribution arrangements vary between these stores.

Why are seeds stored?

Seeds must be stored in a way which maintains their viability for long periods. Seeds left at ambient temperatures and relative humidities will lose their viability quickly whilst seeds stored in conditions of low moisture content and temperature will retain their viability for longer periods. Accessions held in a genebank are valuable and represent plants which are no longer available or which are endangered in their natural environment. These seeds must be conserved in the genebank for use in plant breeding in the future.

When should seeds be placed into store?

As soon as the seed has matured on the plant the slow process of deterioration begins. Therefore the sooner that seeds are placed into store the better. In practical terms this means that seeds collected in the field should be quickly returned to genebanks, processed and placed into store as soon as the cleaning, drying and packaging processes are complete.

How many seeds of each accession should be stored?

The IBPGR Advisory Committee on Seed Storage has recommended that for materials showing little morphological variation (genetically homogeneous) 3000 seeds are acceptable, but 4000 seeds are preferred, to represent each accession. For materials showing a large amount of morphological variation (heterogeneous) an accession should consist of at least 4000 seeds, but 12000 seeds are preferred. These sample sizes are recommended as number of seed but many genebanks would prefer to work with weight. An inter-conversion of numbers and weights is possible from the thousand seed weight of any accession. A list of approximate thousand seed weights of some common species is given in Appendix 2 of Cromarty, Ellis and Roberts (1982).

How should seeds be stored?

The conditions which prolong viability during storage have been well defined for seeds which are tolerant of desiccation. Storage conditions have been recommended by the IBPGR Advisory Committee on Seed Storage. For base collections, seeds of between 3 - 7% moisture content should be stored in sealed containers. Sub-zero temperatures are acceptable, but -18°C or less is preferred. For active collections sealed storage of seeds dried to 7% moisture content or less is recommended at temperatures of less than 15°C. Unsealed storage is not encouraged. In particular, it is <u>not</u> recommended in tropical areas.

Table of thousand seed weights of species in your genebank

Fill in this table to use for future reference:

Species	1000 seed weight	number of seeds/g
.....		
.....		
.....		
.....		
.....		
.....		
.....		
.....		
.....		

STEP 1. CHECK THE NUMBER OF SEEDS IN THE ACCESSION

1. Weigh the seeds of each accession.

2. Convert the weight of seeds to seed number, using the thousand seed weight of each accession for an accurate conversion. An approximate conversion can be done on a species basis by using the approximate weights given in Appendix 2 of Cromarty, Ellis and Roberts (1982).

3. For accessions containing mixtures of genotypes, the sample size should be at least 4000 seeds. For genetically uniform accessions, the sample size should be at least 3000 seeds.

4. If the sample contains more than the required number of seeds, proceed to storage.

5. If the sample contains less than the required number of seeds, either proceed directly to regeneration (See Section X on page 97) or store the seeds temporarily in the genebank and regenerate at the earliest opportunity.

STEP 2. DETERMINE WHERE THE SEEDS SHOULD BE LOCATED

1. Check the inventory data file of the genebank to find the next available space where a container can be located.

2. When seeds from the same regeneration cycle of the same accession are stored in several containers, keep all the containers of the accession together.

3. Make a list of where each accession will be placed.

STEP 3. PLACE IN THE SEED STORE

1. Place the container into the seed store in the listed location.

STEP 4. ENTER THE DATA INTO THE DATA BASE

1. Fill in the data on the location and date of storage of each accession and each container into the data file.

2. Record the date of the next monitoring test for germination in the data file. This date will be determined by the curator after considering the viability and moisture content of the seeds, storage conditions and the IBPGR recommendations.

Notes and Examples	Equipment
An approximate inter-conversion of seed number and weight can be done easily using the thousand seed weight. Example for Sorghum (1000 seed weight = 17 g) 100 g contains: $\frac{1000}{17} \times 100 = 5750$ seeds. These are minimum sample sizes for the start of storage and if both space and seeds are available, more seeds should be held.	Coarse balance
Regenerate as soon as possible. Seeds processed and stored under good conditions will not loose viability before regeneration.	
The storage arrangements will vary among genebanks. The most important point is to know exactly where to locate each accession within the store.	Racks Trays, boxes or drawers Coldroom or freezer
A code can be used to locate an accession within the store. Each unit can be identified by a number or letter in the store. The code can indicate the number or the letter of the freezer, store, rack, basket or drawer, etc. Example: A010201 could be used to indicate the location as: Coldroom: A Rack: 01 Shelf: 02 Box: 01 Colour codes can also be a quick and easy way to locate accessions. A colour can be used for each rack, shelf or species. This both speeds up the work in the coldroom and makes it easy to spot errors. Owing to the very cold temperatures, the faster that one can locate accessions in the cold room the better.	

Arrangement of your seed store

Draw a diagram of and explain the coding system used in your seed store:

SECTION VIII

Monitoring Accessions

What is meant by monitoring?

As previously explained, the viability of the seeds will decrease during storage over long periods. The number of seeds of each accession held will also decrease as seeds are removed for testing and in the case of active collections, when seeds are distributed. Both viability and seed number must be monitored during storage.

Why must accessions be monitored?

Seed viability and number of seeds of each accession in the store must always be known with reasonable accuracy. Only then can accurate decisions on regeneration be made.

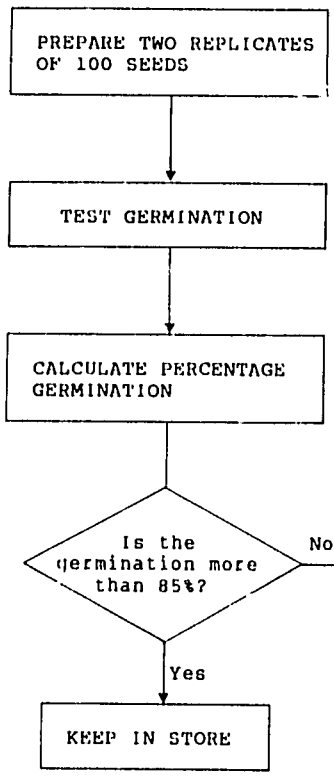
When should seeds be monitored?

The number or weight of seeds in store should be monitored every time seeds are removed from the genebank. Seeds should not be removed if only a few seeds remain. The viability should be monitored at regular intervals. This interval will be determined by the curator depending on the species, viability of the seeds at the start of storage, moisture content and storage conditions. The IBPGR Advisory Committee on Seed Storage recommends that seeds stored under the preferred standards in base collections should be monitored at least every ten years and that those with either poor storage life or poor initial viability in base collections, together with all seeds stored in active collections, should be monitored every five years.

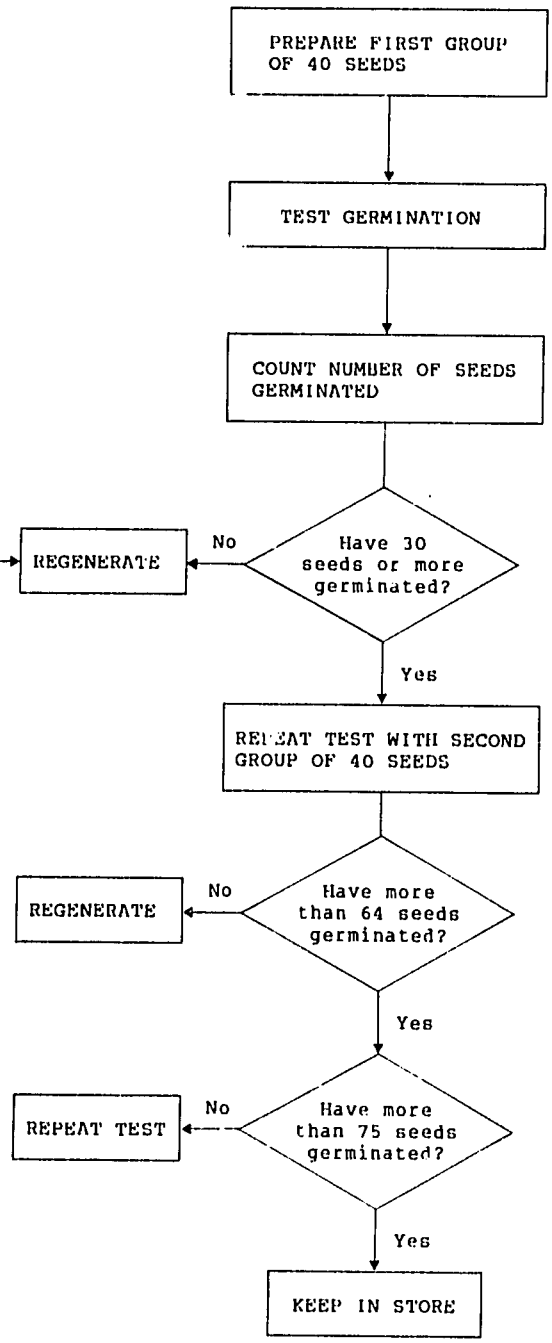
How should accessions be monitored?

The number or weight of seeds can be monitored automatically by checking in the genebank management data file and need not be described further here. The viability can be monitored by doing a fixed sample size germination test as already described in Section V (page 56) or using a sequential method as explained here. Both tests use the same germination conditions and differ only in the number of seeds used for the test and the interpretation of the results. On average the sequential germination test uses fewer seeds and the IBPGR Advisory Committee on Seed Storage has recommended it for monitoring collections in genebanks.

FIXED SAMPLE SIZE GERMINATION TEST



SEQUENTIAL GERMINATION TEST



STEP 1. DETERMINE WHICH ACCESSIONS REQUIRE MONITORING

1. The fact that accessions will be entered into the genebank in an irregular pattern, with many being entered at the same time after harvest or a collecting mission and few accessions being entered at other times, means that monitoring of accessions will also be in an irregular pattern.

2. Every week check the genebank management data file to see which accessions require monitoring during that week.

3. Prepare a list of all accessions by number, which require monitoring during the week.

4. If there are many accessions which require monitoring at the same time, make subgroups of about 20 accessions of similar species which can be handled with the same technique and do these on different days to spread the work.

5. If there are few accessions which require monitoring at the same time, it may be more efficient for them to be grouped and the monitoring done once a month.

STEP 2. PREPARE THE SEEDS FOR TESTING

1. Check each accession number in the management data file to make sure that sufficient seeds are available for the test. If few seeds are available, do not test now because the material will have to be regenerated soon.

2. Make a list of each accession number, its location in the seed store and any data relating to the methods and conditions used for the germination test.

3. Remove the seed containers from the store and leave at room temperature, until the container has warmed up, before opening.

4. Count the number of seeds needed for the test from each accession. Work in a dehumidified room, if possible, and close the containers as soon as the seeds are removed to prevent the seeds absorbing moisture from the air.

STEP 3. DO A GERMINATION TEST

Two methods are recommended by the IBPGR Advisory Committee on Seed Storage:

Fixed sample size germination test

1. Do a fixed sample size germination test using a minimum of 200 seeds sown as two replicates of 100 seeds and follow the techniques already described in Section V (page 56).

2. Calculate the percentage germination from the results of the test.

3. If it is above 90%, accept the test as valid and use this value as the true viability.

4. If the result is 90% or below, repeat the test using a further 200 seeds following the same procedures.

5. Calculate the mean percentage viability from the results of the two tests and use this as the overall test result.

Notes and Examples	Equipment
Do not handle too many accessions at the same time. Availability of staff and equipment determine the number of accessions handled at one time. Samples needing monitoring within one or two weeks can be grouped together for convenience.	
Large containers may take several hours to reach room temperature, but it is important that cans should not be opened before room temperature is reached or condensation may form directly on the seeds and cause changes in moisture content.	
The fixed sample size germination test is done using the standard techniques for the species in your genebank, as already explained in Section V (page 56).	Filter paper, paper towelling or rice straw paper Plastic bags Petri dishes or other containers Water Forceps Incubators Labels Permanent markers Sand Pots or trays Tools for planting

6. Compare the final result of this test with the results of the previous test and decide whether regeneration is necessary, according to the following criteria:

   If the percentage germination has decreased to 85 or less, regenerate the accession.

   If the percentage germination is above 85, the curator will plan the next monitoring test date depending on the germination percentage.

Sequential germination test

1. A sequential germination test uses the same conditions and techniques as already described for the fixed sample size germination test, but uses less seeds for each replicate. The replication is done in a sequential way when necessary and thus not all replicates are tested at the same time.

2. The number of seeds required for each replicate can vary; the more seeds and replicates, the more accurate the test. It is recommended that a sample should consist of 40 seeds per replicate as a minimum.

3. Do a germination test using the conditions and methods as already described in Section V (page 56) using one replicate of 40 seeds.

4. Count the number of seeds germinated.

5. Compare the results of the test with the number germinated in Table 3 (page 88), which shows the germination test plan for the 85% regeneration standard using a sample size of 40 seeds per replicate.

6. Look at the line with the value of 40 in the first column (number of seeds tested).

   (i) If the number of seeds germinated is 29 or less, the accession will require regeneration as explained in Section X (page 97).

   (ii) If the number of seeds germinated is more than 29, then the test will have to be repeated with another sample of 40 seeds exactly as above.

7. It is important that each sample should be treated the same, so that the different samples can be used as replicates.

8. Count the number of seeds germinated in the second test and add this to the result of the number of seeds germinated from the first test. Add the total number of seeds used for both the samples.

9. Compare the results of the test with the number germinated in Table 3 as before. Look at the line with the value equal to the total number of seeds used for all tests (80) in the first column (number of seeds tested).

   (i) If the number of seeds germinated is 64 or less, then the accession will require regeneration.

   (ii) If the number germinated is between 65 and 75, the accession will require testing again with another sample of 40 seeds.

   (iii) If the number germinated is above 75, then the viability of the accession is still above the regeneration standard and the accession does not require regeneration.

10. Continue in this way until either a definite answer as to whether to regenerate or to continue storing the accession is reached or the test has been repeated ten times. The results should then be expressed as percentage germination and a decision as to whether to regenerate taken as already explained in the fixed sample size germination test monitoring method.

Notes and Examples

The sequential test has an advantage in that it uses less seeds but is of a similar accuracy to the fixed sample size germination test. The actual germination test conditions and procedures are the same for both types of germination test, the only difference being in the number of seeds used. The cost of storing germplasm is high; therefore, seeds should not be wasted. The cost of regeneration and the possibility of selection and loss of accessions during regeneration are high. Therefore, test results should be accurate and indicate when regeneration is needed. A balance has to be found between using enough seeds to be accurate and using too many wastefully. This balance is found in the sequential germination test.

If seeds are thought to be of high viability or questionable, it may be simpler to set up the first two sequential replicates together (2 x 40) because as can be seen in Table 3, a second test will probably be necessary. It is easier and quicker to set up two tests together than to repeat the whole process after the results of the first test are known. There is then no delay in getting the results of the second test. When you know prior to testing that the seeds are probably in poor condition, one test is sufficient to show that regeneration must be done.

It is obvious that the test is open-ended and if the answer always fell within the range where the accession required testing again using another sample, a large amount of seeds would have to be used to determine whether regeneration is necessary. Therefore it is suggested that in circumstances where no definite decision can be made regarding regeneration, the test should be terminated when ten samples have been tested. This uses 400 seeds, which is the same as in the fixed sample size germination test for accessions of less than 90% viability. Results can then be converted to percentage germination and a decision on the need for regeneration can be made.

Equipment

Filter paper, paper towelling or rice straw paper
Plastic bags
Petri dishes or other containers
Water
Forceps
Incubators
Labels
Permanent markers
Sand
Pots or trays
Tools for planting

Table 3. Modified sequential germination test plan for 85 percent regeneration standard for groups of 40 seeds

Number of seeds tested	Regenerate if number germinated is less than or equals:	Repeat test if number germinated is between:	Store if number germinated is more than or equals:
40	29	30 - 40	-
80	64	65 - 75	76
120	100	101 - 110	111
160	135	136 - 145	146
200	170	171 - 180	181
240	205	206 - 215	216
280	240	241 - 250	251
320	275	276 - 285	286
360	310	311 - 320	321
400[1/]	340		341

1/ When 400 seeds have been tested, the test can be terminated because enough tests have been done for a practical decision to be made.

Table derived from:

Ellis, R.H., Roberts, E.H. and Whitehead, J. (1980). A new, more economic and accurate approach to monitoring the viability of accessions during storage in seed banks. Pl. Genet. Resources Newsl., 41: 3-18.

SECTION IX

Distribution of Germplasm from Active Collections

What is distribution of germplasm?

This is the supply of seeds of the different accessions in response to requests from plant breeders and research workers. Seeds are distributed only from active collections and not from base collections.

Why is germplasm distributed?

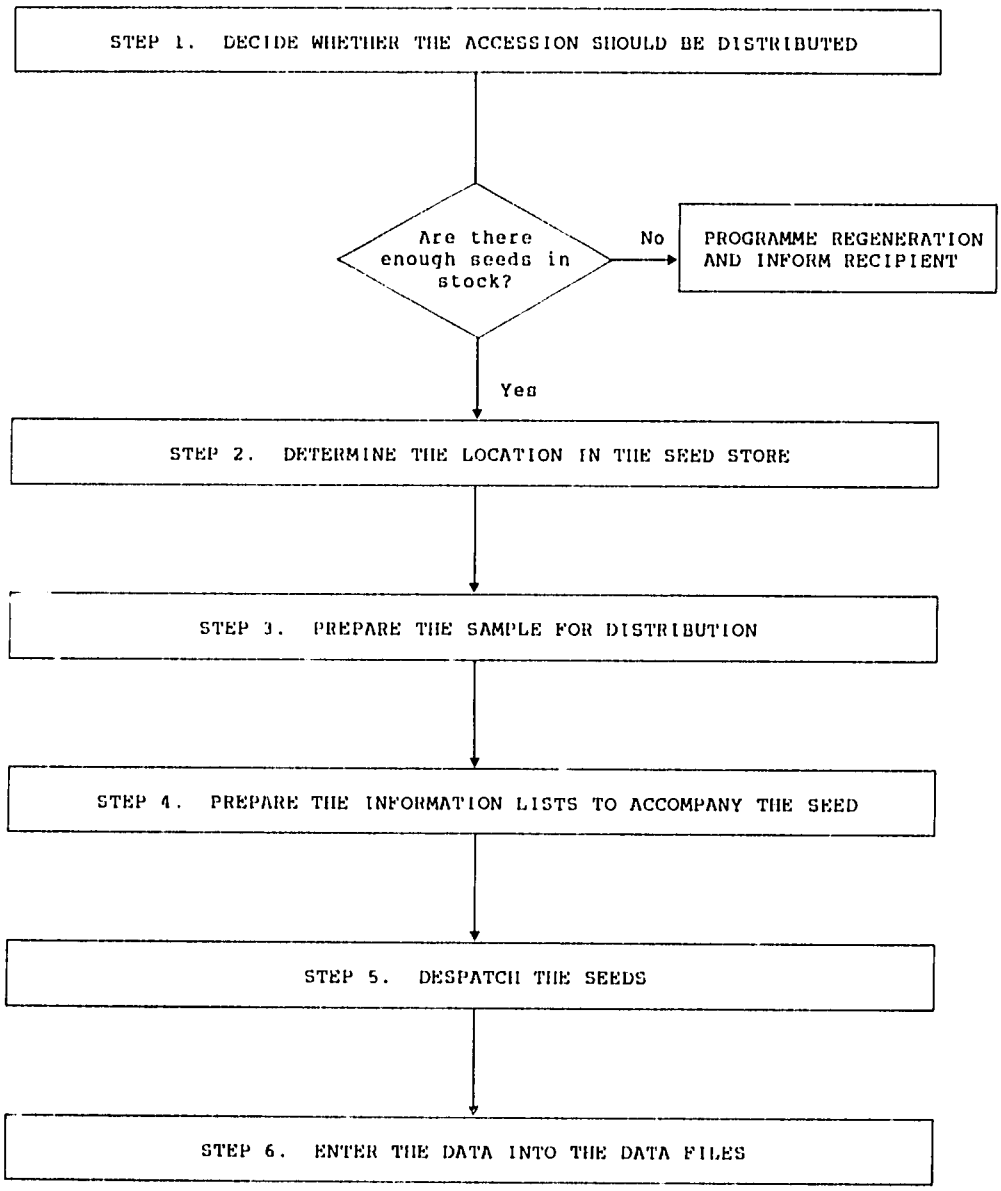
The purpose of keeping the germplasm safe in the genebank is to use it in plant breeding and related activities. It may be distributed for breeding, research, characterization and evaluation or for regeneration of fresh seeds of each accession.

When is germplasm distributed?

Germplasm is distributed freely from the genebank on request. However, considering the high cost of conserving it, the curator must feel that the request is justified.

How is germplasm distributed?

The seeds should be sent out in such a way that they arrive in good condition and are capable of germination. Distribution takes time and environmental conditions during transport can be detrimental to seed quality. Therefore, it is recommended that seeds should be distributed in sealed laminated aluminium foil packets.


STEP 1. DECIDE WHETHER THE ACCESSION SHOULD BE DISTRIBUTED
Are there enough seeds in stock?
No
PROGRAMME REGENERATION AND INFORM RECIPIENT
Yes
STEP 2. DETERMINE THE LOCATION IN THE SEED STORE
STEP 3. PREPARE THE SAMPLE FOR DISTRIBUTION
STEP 4. PREPARE THE INFORMATION LISTS TO ACCOMPANY THE SEED
STEP 5. DESPATCH THE SEEDS
STEP 6. ENTER THE DATA INTO THE DATA FILES

STEP 1. DECIDE WHETHER THE ACCESSION SHOULD BE DISTRIBUTED

1. Check in the data file to see if seeds are available to distribute in response to the request. When the request has been filled there should remain in store a minimum of three times the number of seeds required for one regeneration cycle.

2. When there are not enough seeds, do not distribute. Inform the curator to programme regeneration and to write to the person who has requested the seeds to inform them that the accession cannot be supplied until after regeneration.

STEP 2. DETERMINE THE LOCATION IN THE SEED STORE

1. Check in the genebank management data file the location of the container within the genebank.

STEP 3. PREPARE THE SAMPLE FOR DISTRIBUTION

1. Remove the container from the genebank.

2. Allow the container to warm to room temperature before opening (this may be several hours for large containers).

3. Write the accession number and genus and species of the seeds on two labels, one for the outside and one for inside the seed packet.

4. Work in a dehumidified room, if possible, and close the containers as soon as the seeds are removed to prevent the seeds absorbing moisture from the air.

5. Open the container and use a random sampling technique to weigh or count the required amount or number of seeds quickly. It is suggested that about 50 - 100 seeds should be distributed to fill each request.

6. Place the seeds with one label in an aluminium foil packet, seal and attach the other label to the outside.

7. Record the number or weight of seeds removed from each accession.

STEP 4. PREPARE THE INFORMATION LISTS TO ACCOMPANY THE SEED

1. Prepare a list of all materials sent and their passport, characterization and evaluation data and any relevant genebank management data relating to the germination or dormancy behaviour of the seeds of each accession.

2. For overseas requests, obtain a phytosanitary certificate to accompany the seeds from the relevant authority. If certification of the seeds is not possible, inform the recipient and request him to advise on whether the seeds can be accepted into his country without the certificate.

STEP 5. DESPATCH THE SEEDS

1. Despatch the seeds by air mail or air freight for overseas and by the most direct route for destinations in your country. This will avoid delay and damage to the seeds during transit.

2. Send one copy of the list and phytosanitary certificate with the seeds. Also send copies of the documents separately to the recipient.

Notes and Examples

Seeds should not be distributed from base collections. Care must be taken not to distribute all available seeds of one accession.

As many accessions as possible should be kept available for distribution, although it must be accepted that there will be a temporary delay in distribution until seeds can be regenerated.

Always use random sampling techniques to remove seeds from the container so that the resulting sample will provide a good representation of the original population.

Send as much information as possible with the seeds.

Make sure that the phytosanitory regulations in both your country and the recipient country are met; otherwise seeds may be delayed or confiscated.

Fungicides and insecticides can be used in order to comply with phytosanitory restrictions during distribution because it is expected that the seeds will be sown on arrival.

Equipment

Aluminium foil packets
Sealing machine
Labels
Coarse balance

STEP 6. ENTER THE DATA INTO THE DATA FILES

1. Record the relevant data about the shipment and the accessions sent into the data file on seed distribution.

2. Record the number or weight of seeds removed from each accession in the genebank management data file.

Notes and Examples

It is a good idea to keep a separate file of names and addresses of recipients and the germplasm they have received. These people should then be asked to send data on the accessions back to the genebank.

Flow chart of the distribution process used in your genebank

Please draw a flow chart on this page to show how seeds are distributed from your genebank:

SECTION X

Regeneration of Accessions

What is regeneration?

This is the renewal of an accession by taking a random sample of seeds, sowing and growing the resulting plants under conditions so that the seeds harvested will possess the same characteristics as the original population.

Why is regeneration necessary?

The most important part of conservation of material is that the seeds maintained in the genebank should always produce plants which show the representative characteristics of the accession. If seed viability begins to decrease, then genetic changes can occur in the seeds, thus changing the characteristics of the accession. Therefore renewal of the accession is necessary and must be carried out in the correct way to maintain the characteristics of the original population. Regeneration is also necessary when the number of seeds of the accession in store falls below a critical level.

When are accessions regenerated?

Regeneration is a time consuming and expensive task which is potentially dangerous to accessions because it can allow genetic change and the accession is at the risk of pests and diseases during the growing cycle. Therefore regeneration should be kept to a minimum. It is necessary when the seeds of the accession begin to lose viability, when small seed samples are entered into the genebank or when seed stocks become low due to distribution from active collections. The IBPGR Advisory Committee on Seed Storage recommends that for most species an accession should be regenerated when the seed viability falls below 85%. Regeneration is also required when the number of seeds in an accession falls below the number required to complete 3 regeneration cycles for that species. If there is doubt about the condition of the seeds, their regeneration must take priority; otherwise the sample may be lost. Some very small seed samples may need several cycles of regeneration before sufficient seeds are harvested to reach the recommended sample sizes. FOR INFORMATION ON SAMPLE SIZE, SEE SECTION VII (PAGE 78).

How should regeneration be carried out?

Regeneration is carried out by growing out and harvesting a representative sample of seeds from an accession. The techniques used must ensure that the seeds produced from regeneration are fully representative of the original population. Since species differ in agronomic requirements, breeding systems, compatibility and population structure, the techniques used are crop-specific and will not be discussed in detail in this manual. It is recommended that curators consult with plant breeders and experts on the various crops to identify appropriate methods for regeneration and that these be incorporated into the genebank's regeneration procedures.

Principle factors to consider when selecting a suitable regeneration method

* Suitability of the environment for each accession to ensure that natural selection is kept to a minimum and there will be good seed production.

* Special requirements needed to stimulate germination, to break dormancy and induce flowering (vernalization or photoperiod).

* Optimum number of plants to fully represent the population and ensure that all the characteristics of the accession are maintained.

* Optimum number of plants to ensure that enough seeds are produced to reach the recommended sample size.

* The correct spacing for the plants to ensure optimum seed set and production of good quality seeds.

* The breeding system of the plant (outbreeder or inbreeder) to determine whether isolation is needed.

* The need for an artificial pollination method, either by the introduction of insect pollinators or hand pollination, to ensure seed set.

* The presence of compatibility barriers to pollination which may have to be overcome artificially.

* The sexuality of the plants (dioecious, monoecious or hermaphrodite) to ensure that sufficient male and female plants are available for pollination and good seed production.

* The need to mix approximately equal proportions of seeds from different mother plants to avoid maternal effects from particular plants in the population.

STEP 1. DETERMINE HOW MANY ACCESSIONS CAN BE REGENERATED IN ONE CYCLE

1. Seek advice on the techniques and environmental conditions required for each crop from plant breeders and the curator.

2. Consider the area of the land available for regeneration.

3. Consider the number of plants per plot and their spacing to determine plot size.

4. Consider the area required for isolation and paths between plots.

5. Divide the amount of land available by the amount of land required for one accession including paths. The nearest low whole number is the number of accessions that can be grown in the space available.

6. The curator will consider the work involved and the staff available and decide how many accessions to handle.

STEP 2. DECIDE WHICH ACCESSIONS TO REGENERATE

1. Make a list of all accessions with a seed viability of less than 85%.

2. Make a list of new accessions which contain less than the recommended number of seeds for storage in the genebank (for details see Section VII page 78).

3. Make a list of all accessions with the number of seeds remaining less than three times the number of seeds used for regeneration of that species.

4. The curator will decide which accessions have priority. As a general rule first priority should be given to the accessions with the lowest seed viability, second priority to any accessions which are required urgently from either list and lastly to accessions with high viability but few seeds.

5. Make a list of the accessions which the curator has decided to regenerate in this cycle and a second list of other accessions which are awaiting regeneration in the future.

6. Record the location of each accession in the genebank from the list of those to be regenerated.

STEP 3. DETERMINE WHERE TO GROW THE MATERIAL

1. Check the passport data to find which environmental conditions are needed for that accession.

2. Check the number of seeds that are available.

3. If few seeds are available and the accession is sensitive to the prevailing environment, grow under controlled conditions or in a glasshouse.

4. If enough seeds are available and the accession is well adapted to the outdoor climate or special conditions are not available, grow in the field.

STEP 4. PREPARE THE PLANTING PLAN

1. Draw a scale map of the area to be used.

2. Mark this plan off into plots of the correct size for each species, allowing room for isolation blocks, inter-planting of species and paths for access.

Notes and Examples

Equipment

Calculator/graph paper
Packets
Harvest bags/packets
Labels
Stakes
Isolation bags or cages

Do not handle too much material at one time - it is better to handle fewer accessions well than many accessions badly. Generally speaking, seeds stored in the genebank can usually be kept for another year without greatly decreasing seed viability.

Select the right environment for the accession. Take special note of day-length requirements in certain crops or they may not flower. In some cases where seed dormancy is present it may be necessary to treat the seeds first before sowing in the field.

3. Fill in the accession numbers from the list already prepared into the appropriate plots, taking account of any isolation distances, species and maturity differences necessary to prevent crossing between plots.

STEP 5. PREPARE THE SEEDS FOR PLANTING

1. Remove the container from the genebank.

2. Allow the container to warm to room temperature before opening. This may be several hours for large containers.

3. Write the accession number and genus and species of the seeds on the outside of a seed packet.

4. Open the container and quickly weigh or count the required number of seeds already decided as being appropriate for the regeneration of accessions of that species, but do not use more than half of the seeds which are available for that accession. Work in a dehumidified room, if possible and close the containers as soon as the seeds are removed to prevent the seeds absorbing moisture from the air.

5. Place the seeds in the labelled paper packet and seal.

6. Record the number or weight of seeds removed from each accession.

STEP 6. PROCEED WITH THE REGENERATION

1. Follow the planting plan using techniques outlined by the breeders and curator to ensure that regeneration is done well and no crossing occurs between accessions.

STEP 7. HARVEST THE NEW SEEDS

1. Harvest each accession separately when the seeds are fully mature.

2. Mix the same weight of seeds from all the plants of one accession to minimize maternal effects and still keep as much variation as possible within the sample. Make sure that population samples are not divided into lines but keep the accession complete.

STEP 8. PROCESS THE SEEDS AND ENTER NEW SEEDS INTO THE GENEBANK

1. Carry out seed cleaning (Section II, page 15) and measure the weight or number of true seeds harvested.

2. Do not mix the seeds from the different regeneration cycles.

3. Use the seeds from earlier regeneration cycles to supply seed requests.

4. Regeneration may need to be carried out several times to reach the recommended sample size for genebanks (Section VII, page 78).

5. Use the other half of the original sample for the second regeneration and representative samples from these two for further regeneration.

STEP 9. ENTER THE DATA INTO THE DATA FILES

1. Enter all inventory data referring to the new seed into the appropriate data files.

2. Characterization can also be carried out during regeneration and this type of data should be entered into the appropriate data file.

Notes and Examples

Always use random sampling techniques to remove seeds from the container so that the resulting sample will provide a good representation of the original population.

Make sure that the population is kept whole and not split into lines. If lines are required for plant breeding needs, it is possible to keep these separate in a different programme, but the population should also be maintained intact.

It is better to go back to the original seeds to sow for regeneration, if these seeds still have a high viability, because each time that seeds are regenerated the characteristics of the population can be changed from recombination of genes and selection. Therefore it is better to have seeds from the least number of regeneration cycles in order that genetic integrity is maintained.

Table of regeneration procedures used in your genebank

Fill in this table to use as your future personal guide:

Species	Number of Plants	Spacing	Breeding System	Isolation	Pollination Method
.....					
.....					
.....					
.....					
.....					
.....					
.....					
.....					
.....					

APPENDICES

APPENDIX I

Further reading

Cromarty, A.S., Ellis, R.H. and Roberts, E.H. 1982 The Design of Seed Storage Facilities for Genetic Conservation. IBPGR, Rome.

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ISTA 1981 Amendments to the International Rules for Seed Testing 1976. ISTA, Zurich.

ISTA 1985 International rules for seed testing, rules 1985. Seed Sci. and Technol. (in press).

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APPENDIX II

Glossary of technical terms

Accession	A sample (made up of seeds of a variety, strain or a population) maintained at a genetic resources centre for conservation or use.
Accession number	A unique identifier for accessions that is assigned by the curator when the accession is entered into the collection. Once assigned this number should never be assigned to another accession in the collection and should not be used again.
Characterization	The recording of those characters which are highly heritable, can be easily seen by eye and are expressed in all environments.
Collector's number	Original number assigned y the collector of the sample.
Data base	A collection of interrelated data files which can serve as an information source for many users.
Data file	A collection of data which are accessible as a single entity.
Donor	The person or institution which has donated the seeds.
Dormancy	The condition of a viable seed which prevents germination when the conditions normally considered to be suitable for germination are provided.
Equilibrium moisture content	The moisture content at which a seed is in equilibrium with the relative humidity of the surrounding air.
Evaluation	The recording of those characters that are important for the use of a crop.
Genebank	A centre conserving seeds, which are considered as genetic resources, under suitable conditions to prolong the life of the seeds.
Genetic variability	Variation in the genetic structure of the species.
Germination	The initial stages of growth of a seed into a seedling.
Hermetic seal	An air-tight seal used to close containers.
Inventory	A list of samples (and of their characteristics) that are stored in the genebank.
Laminated aluminium foil packets	Packets constructed of a laminate consisting of an inner layer of polyethylene, a middle layer of aluminium foil and an outer layer of polyester.
Passport data	The data referring to the identification and origin of an accession.

Phytosanitory certificate	A certificate provided by Government Plant Health Personnel to certify that the seed material is free from pests and diseases.
Population sample	A sample of individuals that are representative of the population from which they were drawn.
Random sample	A sample which is drawn by random methods from a larger bulk.
Recipient	The person who receives the material or information.
Regeneration standard	The percentage seed viability at or below which the accession must be regenerated as soon as possible to produce fresh seeds.
Relative humidity	The ratio of the weight of water vapour present in the atmosphere to the weight which would saturate the atmosphere at that temperature.
Silica gel	An inert chemical which absorbs water from its surroundings and will give up this water again by evaporation when heated.
Storage life	The number of years that a seed can be stored before seed death occurs.

APPENDIX III

Suitable equipment for use in genebanks

Equipment	Specifications
Specialized equipment	
Seed blower	Commercially available or custom built. The column should measure about 60 cm with a strong air flow
Analytical balance	Single pan balance with a range of 0 - 55 g and 0.1 mg readability. If available an electronic balance is better
Binocular stereo microscope	Research microscope with a range of magnifications from 0.5 to 240x
Cans - screw top or sealed	Screw top with inner seal or good quality can which may be hermetically sealed. The size and shape may be varied to meet the needs of the genebank
Coarse balance	Top pan balance with a range of 0 - 1600 g and a readability of 0.01 g. If available an electronic balance is better
Computer and software	Micro-computer with printer and commercial data base package
Dehumidifier dryer	A sorption dryer capable of attaining 10 - 15% relative humidity at 15°C in an enclosed space
Freezers	Good quality domestic chest freezer units capable of holding temperatures of -20°C for long periods
Glass bottles	Screw top with inner seal or sealed tops. The size and shape can be varied to meet the needs of the genebank
Light table	Transparent screen with lighting from below of any convenient size
Incubators	Cyclic illuminated, refrigerated incubators with a range of 0 to 50°C temperature (to cope with germination tests, vernalization and heat treatments to break dormancy)
Laminated aluminium foil packets	Made of a middle layer of aluminium of 12 μm and 4 g/m^2 laquer, and an inner layer of 63 g/m^2 polythene. Any size can be used to meet the needs of the genebank
Oven	Fan-circulated oven with a temperature range of 30 - 200°C and a quick heating and recovery time to constant temperature
Sealing machines	Specific to the type of container
Seed grinder	Laboratory mill for grinding wet and dry samples with screens of 0.5 - 4 mm perforations
Sieves	Set of metal sieves with aperture dimensions of 0.5, 1, 2, 2.8, 4, 5.6, 8 and 16 mm and a cover and receiver set
Threshing machine	Mini threshing machine for small samples

Equipment	Specifications
General laboratory equipment	
Bamboo baskets	Locally available
Bottles with air-tight lids	Locally available
Calculator	Locally available
Cans with air-tight lids	Locally available
Cloths	Locally available
Desiccators	Large glass desiccators
Forceps	Locally available
Heat resistant dishes	Dishes of 2.5 cm diameter or less made of aluminium, pyrex glass or good quality aluminium foil
Low temperature thermometers	To read from -30 to 50°C at 0.5°C intervals
Maximum minimum thermometer	To read from -20 to 55°C
Petri dishes	Pyrex glass petri dishes in a range of sizes of 52, 95 and 140 mm
Planting tools	Locally available
Plastic bowls	Locally available
Plastic boxes	Locally available
Porous containers	Locally available
Pots or trays	Locally available
Scoops	Locally available
Silica gel	Self-indicating silica gel beads for drying
Small brushes	Locally available
Small containers or boxes	Locally available
Spatulas	Locally available
Spoons	Locally available
Thermohygrometer or aspirated hygrometer	To read from 5 - 100% relative humidity with 1% accuracy
Tongs	Locally available
Trays	Locally available

Equipment	Specifications
Consumables	
Absorbent paper	Pure and free of chemicals
Aluminium foil	Good quality domestic kitchen foil
File cards	Locally available
Graph paper	Locally available
Isolation bags	Locally available
Notebooks	Locally available
Paper labels	Locally available
Paper packets	Locally available
Paper towelling	Pure and free of chemicals
Pencils	Locally available
Pens	Locally available
Permanent markers	Locally available
Plastic bags	Locally available
Rice straw paper	Locally available
Sand	Locally available
Wood labels	Locally available

APPENDIX IV

Experts to contact for advice

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