Bioversity International

Handbooks for Genebanks No. 8

Manual of Seed Handling in Genebanks

N. Kameswara Rao, Jean Hanson, M. Ehsan Dulloo, Kakoli Ghosh, David Nowell and Michael Larinde

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Manual of
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Bioversity International is an independent international scientific organization that seeks to improve the well-being of present and future generations of people by enhancing conservation and the deployment of agricultural biodiversity on farms and in forests. It is one of 15 centres supported by the Consultative Group on International Agricultural Research (CGIAR), an association of public and private members who support efforts to mobilize cutting-edge science to reduce hunger and poverty, improve human nutrition and health, and protect the environment. Bioversity has its headquarters in Maccarese, near Rome, Italy, with offices in more than 20 other countries worldwide. The Institute operates through four programmes: Diversity for Livelihoods, Understanding and Managing Biodiversity, Global Partnerships, and Commodities for Livelihoods.

The international status of Bioversity is conferred under an Establishment Agreement which, by January 2006, had been signed by the Governments of Algeria, Australia, Belgium, Benin, Bolivia, Brazil, Burkina Faso, Cameroon, Chile, China, Congo, Costa Rica, Côte d'Ivoire, Cyprus, Czech Republic, Denmark, Ecuador, Egypt, Greece, Guinea, Hungary, India, Indonesia, Iran, Israel, Italy, Jordan, Kenya, Malaysia, Mali, Mauritania, Morocco, Norway, Pakistan, Panama, Peru, Poland, Portugal, Romania, Russia, Senegal, Slovakia, Sudan, Switzerland, Syria, Tunisia, Turkey, Uganda and Ukraine.

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A NOTE TO READERS

Bioversity International's Handbooks for Genebanks are intended to provide practical information to genebank curators and others working in genebanks. To facilitate their use their binding allows them to be kept open on a desk or bench, while the wide margins and 'Notes' pages provide space for readers to make notes or annotate the text.

Bioversity welcomes feedback from readers on the content and format of the handbook for possible future revisions.

PARTNERS IN THIS PUBLICATION

The International Livestock Research Institute (ILRI) works at the crossroads of livestock and poverty, bringing high-quality science and capacity-building to bear on poverty reduction and sustainable development. ILRI is a non-profit-making organization with headquarters in Nairobi, Kenya, conducting research in Africa, Asia and Latin America and the Caribbean, with offices in East, West and Southern Africa, South and Southeast Asia, China and Central America. As one of the 15 Future Harvest centres of the Consultative Group on International Agricultural Research (CGIAR), ILRI's strategy focuses on three livestock-mediated pathways out of poverty: (1) securing the assets of the poor, (2) improving the productivity of their livestock systems and (3) improving their market opportunities in the face of rapidly changing market channels and demands. ILRI's research portfolio comprises four issue-oriented themes: Targeting research and development opportunities; Improving market opportunities; Using biotechnology to secure livestock assets; and People, livestock and the environment.

The Food and Agriculture Organization of the United Nations leads international efforts to defeat hunger. Serving both developed and developing countries, FAO acts as a neutral forum where all nations meet as equals to negotiate agreements and debate policy. FAO is also a source of knowledge and information. FAO helps developing countries and countries in transition modernize and improve agriculture, forestry and fisheries practices and ensure good nutrition for all. Since its founding in 1945, FAO has focused special attention on developing rural areas, home to 70 percent of the world's poor and hungry people. FAO's activities comprise four main areas: putting information within reach; sharing policy expertise; providing a meeting place for nations; bringing knowledge to the field.

The Technical Centre for Agricultural and Rural Cooperation (CTA) was established in 1983 under the Lomé Convention between the ACP (African, Caribbean and Pacific) Group of States and the European Union Member States. Since 2000, it has operated within the framework of the ACP-EC Cotonou Agreement.

CTA's tasks are to develop and provide services that improve access to information for agricultural and rural development, and to strengthen the capacity of ACP countries to produce, acquire,

exchange and utilise information in this area. CTA's programmes are designed to: provide a wide range of information products and services and enhance awareness of relevant information sources; promote the integrated use of appropriate communication channels and intensify contacts and information exchange (particularly intra-ACP); and develop ACP capacity to generate and manage agricultural information and to formulate ICM strategies, including those relevant to science and technology. CTA's work incorporates new developments in methodologies and cross-cutting issues such as gender and social capital.

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FOREWORD

Genebanks are the storehouses of plant genetic resources, providing the raw material for the improvement of crops. They play a key role in contributing to the sustainable development of agriculture, helping to increase food production and thus to overcome hunger and poverty. Inherent resistance to pests and diseases can be bred into crop plants, reducing the need to use chemicals that can have deleterious effects on farmers and the environment. The seeds contained in genebanks are a vital and irreplaceable resource, a heritage which must be conserved to provide future agricultural options in a world facing climate change and other unforeseen challenges. The sustainable conservation of genetic resources depends on effective actions by genebank staff, who play a critical role in ensuring that germplasm is effectively and efficiently conserved. They need to apply proper procedures for handling seeds to ensure their survival and availability to present and future generations.

The practical manual on Procedures for Handling Seed in Genebanks (Hanson, 1985), published by the International Board for Plant Genetic Resources (IBPGR), a predecessor of Bioversity International, has helped genebank curators and technicians in seed conservation in the past. Research over recent decades has yielded advances in knowledge regarding seed physiology and seed-storage behaviour. The Convention on Biological Diversity (CBD) in 1992, the International Treaty on Plant Genetic Resources for Food and Agriculture (PGRFA) in 2004 and related agreements have changed the global framework of germplasm ownership and benefit-sharing. The development of genetically modified organisms (GMOs) and associated controversies have important implications for the ways genebanks manage their germplasm, notably to prevent the unintentional introgression of exotic genes, including transgenes. All of these new opportunities and challenges called for an update of the 1985 handbook for genebanks. This manual addresses these recent changes, and is intended to ensure that seed handling in genebanks meets today's requirements. The new manual is complemented by an interactive self-learning module, found on the CD ROM included in this package. The manual and self-learning module are intended to help address the challenges associated with the shortage and frequent turnover of qualified genebank staff, particularly in developing countries.

The manual and its accompanying self-learning module provide detailed procedural guidelines and lessons for staff who do not

have the opportunity to attend courses on seed conservation and genebank management. Using the manual and its module, genebank staff can learn on their own about the different tasks of the genebank and also have a quick reference to essential genebank procedures. With this publication we hope to contribute to ensuring that genebank staff around the world will maintain to high standards of survival and quality the germplasm under their care.

Laura Snook
Programme Director
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PREFACE

Proper seed-handling procedures in genebanks are fundamental to the long-term, cost-effective and efficient conservation of plant genetic resources. They ensure that seeds placed in storage are of the highest quality and achieve maximum longevity. The aim of genebanks is to maintain high-viability accessions for long periods. Advances in our knowledge of seed biology over the past decades have led to an increased understanding of seed physiology and seed-storage behaviour, which makes seeds the easiest and most convenient means of long-term conservation. This has led to the development of techniques for proper seed-handling and preparation for storage in genebanks.

In the 1980s, the International Board for Plant Genetic Resources (IBPGR), a predecessor of Bioversity International, commissioned a series of handbooks for genebanks, including 'Practical Manuals for Genebanks No. 1: Procedures for Handling Seeds in Genebanks' by Jean Hanson (1985), to help genebank curators and technicians in seed conservation. This out-of-print publication is still a standard reference for genebank work and remains one of few sources of practical information for genebank curators and technicians. In recent years, very few books have been published on this subject. The few excellent scientific publications on this topic are often too complex for use by the average technician, particularly in developing countries. This manual is an update of the earlier practical manual for new genebank staff members who have not received formal training. It benefits from advances in seed technology, seed storage and management techniques over the last two decades; modern communication technology is used to make it more widely accessible.

The need for this product was highlighted in a recent assessment of training and capacity needs for national programmes by Bioversity and the Food and Agriculture Organization of the United Nations (FAO). As expressed by partners during various meetings and workshops, lack of trained staff is a major constraint for many genebanks in conserving germplasm efficiently and effectively. In developing countries, staff employed by genebanks for routine operations lack basic knowledge of seed physiology and good genebank management practices; rapid staff turnover also aggravates the problem. This simple and practical manual will assist genebank staff in their day-to-day work.

This publication only addresses storage of orthodox seeds—that is, seeds that can be dried to low moisture content and kept at

sub-zero temperatures in genebanks. The major activities and procedures of seed-genebank operation are generally the same across genebanks, although they may vary in content and scope.

Since the first edition of the practical manual in 1985, there have been several new developments that warrant a revision of genebank procedures. The entry into force of the Convention on Biological Diversity (CBD) in 1992 and the International Treaty on Plant Genetic Resources for Food and Agriculture (PGRFA) in 2004 have changed the perception of germplasm ownership and benefit-sharing in the world. These agreements provide new guiding principles for the acquisition, conservation and utilization of biodiversity, and influence the way genebanks carry out their work. Under the CBD and the International Treaty on PGRFA, it is recognized that biodiversity is the sovereign right of nations; germplasm collection must be undertaken with prior informed consent; and acquisition of germplasm is subject to mutually agreed terms and multilateral or bilateral transfer agreements.

In addition, the Global Crop Diversity Trust has been established to support the development and funding of crop diversity conservation around the world. The Trust is helping to maintain collections that are critical for food security and sustainable development. In order to receive support from the Trust, genebanks must satisfy a number of eligibility criteria, one of which is that recipients have the human resources and management systems needed to maintain plant genetic resources and can demonstrate conformity with agreed scientific and technical standards of management. This calls for guidelines to assist genebanks in maintaining high standards of management.

A new and controversial development that is likely to affect germplasm management is the handling of genetically modified organisms (GMOs). Genebanks are now expected to take proactive steps to prevent the unintentional introgression of exotic genes, including transgenes, not already present in samples conserved in their genebanks. For this reason, the Genetic Resources Policy Committee (GRPC) of the Consultative Group on International Agricultural Research (CGIAR) has developed guiding principles that address the risks of introducing transgenes into collections, particularly at critical points in genebank procedures. These principles and the changes in protocol needed to implement them are elaborated in this new edition.

The publication of this manual is a joint initiative of Bioversity International (formerly IPGRI), the International Livestock Research

Institute (ILRI) and FAO, sponsored in part by the Technical Centre for Agricultural and Rural Cooperation ACP-EU (CTA). The manual is intended for genebank staff, especially technicians who handle orthodox seeds, and attempts to give simple explanations of procedures for the day-to-day management of seed-handing in genebanks. To increase this manual's value for training and capacity development, the authors have adapted it to create an interactive self-learning module, which will be published by Bioversity International with support from CTA as a complementary tool on the Web and on CD-ROM.

It is important to note that this manual focuses solely on seed-handling procedures and does not cover documentation, collecting or characterization procedures in great detail; these subjects are well covered by other publications. Readers should consult the literature to gain a better understanding of these important genebank activities.

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1. Introduction

1. INTRODUCTION

For plant genetic resources, the physiological storage behaviour and inherent longevity of each species' seeds dictate the mode of conservation to use. Seed storage is the preferred method for 90% of the six million accessions conserved in ex situ collections worldwide because it is practical and economical. It is the principal conservation method for species producing orthodox seeds that withstand desiccation to low moisture contents and storage at very low temperatures. Most arable and forage species, and many tree species, produce seeds in this category. Techniques for conserving orthodox seeds have been perfected for several decades. These involve drying seeds to low moisture contents (3–7% fresh weight, depending on the species) and storing them in hermetically-sealed containers at low temperature, preferably -18°C or cooler (FAO/IPGRI, 1994).

Several important tropical and subtropical tree species produce seeds that do not survive desiccation and cannot tolerate low temperatures, and which are therefore not easy to store; these are known as recalcitrant seeds. Techniques exist for storing some recalcitrant seeds, but the seeds are usually short-lived and each species requires its own method. A third category of seeds showing intermediate behaviour has also been recognized: these seeds tolerate combinations of desiccation and low temperatures. There is, in fact, a gradient from orthodox to recalcitrant, with no sharp boundaries between categories. Although research has been conducted to overcome problems associated with seed conservation, little progress has been made beyond short-term storage of non-orthodox seeds.

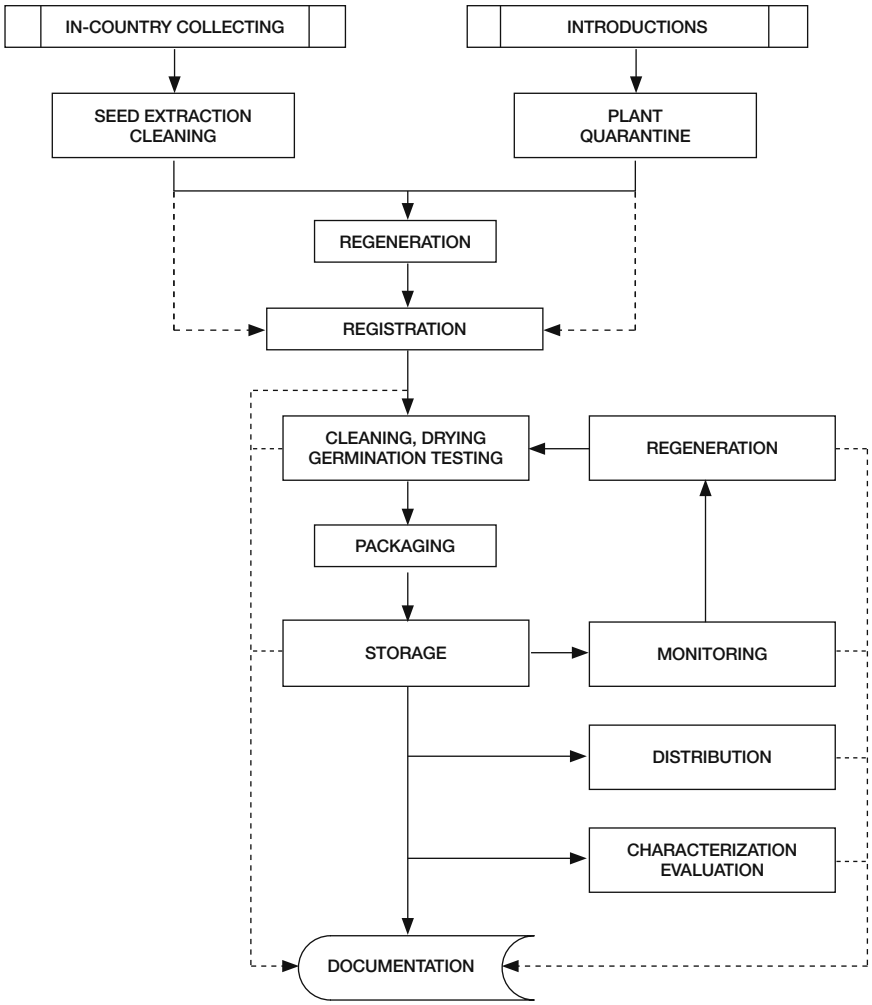
This publication addresses the storage of orthodox seeds in genebanks. The basic operations of a seed genebank include assemblage, processing, conservation, regeneration and distribution of germplasm. Major activities and procedures for genebank operation are generally the same across genebanks, although they may

vary somewhat. Seed genebanks can be very specialized; for example, the International Rice Genebank in Los Baños, Philippines only conserves rice and its wild relatives. Most national seed genebanks, however, store all types of crop seeds. Maintenance of seed viability and genetic integrity remain the cornerstones of genebank management. The quality and sustainability of any genetic conservation effort depends on how seeds are processed and conserved. Inappropriate procedures for seed handling lead to accelerated deterioration, making conservation more expensive.

Genebank operation involves a series of complex and inter-dependent activities (see Flowchart 1.1). Assemblage of germplasm through collection in areas of known genetic diversity or through donations from other centres is the first step towards *ex situ* conservation of crop diversity. Following receipt at a genebank, seed samples are registered and added to the collection, provided they meet required standards for seed quality, quantity and accompanying information. The procedure for integrating an accession into a genebank involves cleaning, moisture determination, drying, viability-testing and packaging. Germplasm accessions must be maintained with a high proportion of viable seeds; this involves storage under appropriate conditions, periodic monitoring of seeds for viability and regeneration when the situation warrants it. Regeneration should be conducted under optimal conditions to maintain genetic integrity and maximize longevity. To minimize genetic drift, adequate numbers of plants should be grown and equally sampled. The genetic integrity of cross-pollinating crops must also be maintained by controlled pollination or isolation. Seeds should be harvested after they have reached the point of physiological maturity and conditioned or 'processed' under optimal conditions to ensure high viability and availability for storage. Good climatic conditions during the post-maturation pre-harvest periods are also vital to the quality of seeds at harvest time: dry weather accelerates drying of seeds on the plant to a moisture content that is favourable for handling while moist weather (with high relative humidity) delays drying of seeds on the plant, leading to seed deterioration even before harvest.

Most genebanks are mandated to distribute germplasm to users. Germplasm accessions are usually distributed using material transfer agreements (MTAs) that define the terms and conditions for use, and provisions for the sharing of benefits arising from germplasm. In the first meeting of the Governing Body of the International Treaty on Plant Genetic Resources for Food and Agriculture in June 2006, a standard material transfer agreement (SMTA) was formalized; the SMTA provides a uniform contract for use in all exchanges of germplasm of the species included in Annex I of the Treaty. It

Flowchart 1.1. General sequence of operations in a seed genebank.



includes specific terms governing access and benefit-sharing, facilitating germplasm exchange around the world.

Genetic resources work involves the management of large quantities of information, requiring documentation and storage in information management systems. Information management is not covered in this manual, but the reader should consult the guidebook for genetic resources documentation by Painting et al. (1993).

Genebank management requires creative and adaptive decision-making. In view of the increasing pressure on genebanks to improve cost efficiency and effectiveness, there is a need to develop coherent management strategies. Important elements of management at both the genebank and the collection levels are analyzed, and options for more efficient and cost-effective management are discussed, in a recent publication by Engels and Visser (2003).

Further reading

Engels, J.M. and Visser, L. (eds.). 2003. A guide to effective management of germplasm collections. IPGRI Handbook for Genebanks No. 6. IPGRI, Rome, Italy.

FAO/IPGRI. 1994. Genebank standards. FAO and IPGRI, Rome, Italy.

Painting, K.A., Perry, M.C., Denning, R.A. and Ayad, W.G. 1993. Guidebook for genetic resources documentation: A self-teaching approach to the understanding, analysis and development of genetic resources documentation. IBPGR, Rome, Italy.

2. Acquisition

2. GERMPLASM ACQUISITION AND REGISTRATION

2.1 Germplasm acquisition

What is germplasm acquisition?

Germplasm acquisition involves obtaining genetic material of a species mandated for conservation in a genebank. It is the initial step in conservation of genetic resources.

Why is it done?

The main reason for acquiring germplasm is to ensure that sufficient diversity is available to meet current and future needs. Reasons for acquisition include:

- genetic erosion: when the threat of genetic diversity loss is present in a particular area and *in situ* conservation is not possible;
- gap-filling: when diversity is missing or insufficiently represented in an existing collection;
- need-based acquisition: when germplasm is needed for breeding, research or development work; and
- opportunistic acquisition: the unplanned, fortuitous collecting of non-target species as opportunities arise.

How is it done?

Germplasm is acquired by:

a. collecting it from farmers' fields, wild habitats or markets, particularly in known centres of diversity; and
b. securing materials of interest through correspondence and exchange with other plant-introduction centres, genebanks, individual scientists, private growers, seed companies or other germplasm suppliers.

Germplasm acquisition policy

Genebanks should have clear policies on acquisition so that the volume of material acquired is within the limits of each genebank's management capacity. When storage space or resources to maintain collections are limited, germplasm should be acquired based on priority.

Prioritization

Acquisition of germplasm should be based on its value or perceived threat of extinction. Value can be assessed as the usefulness of traits and adaptation to unique environments. Landraces, primitive cultivars and wild and weedy species should receive high priority for acquisition, followed by genetic stocks, elite breeding material and obsolete and modern varieties. Consider the availability of resources for management before acquiring wild taxa.

The Convention on Biological Diversity (CBD) and the International Treaty on Plant Genetic Resources for Food and Agriculture (PGRFA) provide the frameworks for acquisition and utilization of germplasm. Collecting is bound by the CBD, which covers access with prior informed consent under mutually agreed-upon terms and benefit-sharing. The International Treaty on PGRFA specifically refers to crop species listed in Annex I of the Treaty, which participating countries have identified as important for inclusion in a multilateral system of access. Access to germplasm under both these international instruments is now governed by the Standard Material Transfer Agreement (SMTA), which was adopted by the Governing Body of the Treaty; the terms of the SMTA cover both access to genetic materials and the benefits derived from them, and should be taken into account during collection and exchange of material.

A. Germplasm acquisition by collecting

Planning and implementation of germplasm-collecting has received in-depth coverage in publications by Guarino et al. (1995) and Smith et al. (2003). Genebank staff should refer to these publications for additional information.

Timing of seed collection

Ideally, seeds should be collected at optimum maturity when seed vigour, desiccation tolerance and longevity are expected to be highest. While it is difficult to monitor these traits in the field, changes in fruit colour, seed colour or black layer formation (in cereals) can be used as visual indicators to make preliminary assessments of optimal seed maturity. These changes correlate well with achievement of mass maturity, although not necessarily with maximum longevity. Nevertheless, they serve as useful indicators for germplasm collectors. Seed dispersal is also a good practical marker of seed maturity.

Fruit colour

In fleshy fruits, colour changes — usually from green to yellow, brown or red — occur with maturity.

- In tomato, red fruit colour indicates that most seeds are at maximum longevity. Seeds from green, yellow/pink or over-ripened fruits are likely to be immature or over-mature and of poor quality.
- In *Cucurbita moschata*, a change in fruit colour from green to yellow-brown and a straw-coloured peduncle indicate high seed vigour.
- In *Capsicum annuum*, seed vigour improves as fruit colour changes from green to red with a few green flecks and then to intense red.
- In *Brassica oleracea*, fruit (silique) colour changes from green to yellow, and in soya bean and many other legumes, it changes from green to yellow-brown and then to brown as the seed matures.

Seed colour

In many dry fruits, seed colour changes from green to yellow or brown as seeds mature:

- In soya bean, seed colour changes from green to yellow-green to yellow.
- In *Sesbania bispinosa*, seed coat colour changes from yellow to olive green and then to greenish brown.

Black layer formation

In cereals such as maize and sorghum, maturity coincides with the formation of a black or brown abscission layer, referred to as the 'black layer'. Maturity is also indicated by drying of the husks and lower leaves.

- The black layer is located on the kernel base at the point of attachment to the cob on the opposite side of the embryo (maize) or at the tip of the grain (sorghum and millet).
- The black layer may be found by gently scraping away the seed coat to expose the abscission layer.

Variation in seed maturity

Germplasm collectors often encounter variation in seed maturity as a result of differences in flowering time between plants and within a single inflorescence on individual plants. This can be overcome by collecting fruits of uniform maturity—provided some markers and sufficient time are available.

Containers for collecting samples

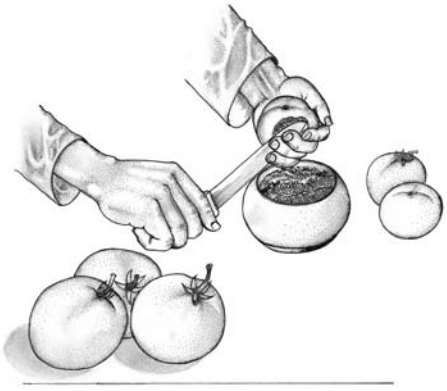
- Use paper bags for collecting seeds.
- Use cloth bags that allow circulation of air (such as muslin bags) for collecting panicles or dry fruits.
- Use open containers, such as baskets made of wire or bamboo or tubs to collect fleshy fruits.
- Ensure that fruits are not squashed.
- During transport, do not let fruits become too hot and ferment.

- Nylon-net bags are also very useful for collecting samples as they allow air to circulate freely. Besides their use for collecting seeds, pods and fruits, they can be used for seed extraction and for drying extracted seeds. They are available in a range of mesh sizes.

Processing of seeds in the field

Newly collected seeds often have high seed-moisture contents (10–20%) and are at risk of deteriorating from contamination with fungi and bacteria. Moist fruits and seeds have high respiration rates and if oxygen is depleted because of inadequate aeration, fermentation sets in. Both respiration and fermentation create heat, resulting in damage to the collected material. When collecting missions are particularly long, pre-cleaning, extraction of seeds and drying in the field becomes necessary in order to reduce bulk and weight during transportation, remove contaminants and bring seed moisture content to a safe level.

- Employ only manual methods for cleaning and seed extraction in order to maintain viability.
- If seeds are collected with surface moisture, dry them first in shade or a well-ventilated room by spreading them on newspaper or blotting paper before transferring them to cloth or paper bags.
- Seeds from dry dehiscent fruits (such as okra, rapeseed and sesame) can be extracted by spreading the fruits on a tarpaulin under shade.
- Mature fruits split open and release their seeds as they dry. Sometimes, additional impact such as raking or shaking is needed.
- Remove empty fruits and debris, and transfer the seeds into cotton, nylon-net or paper bags.
- With pulpy fruits (such as tomato and cucumber), extract the seeds carefully by hand, wash them under running water to remove pulp and mucilage, spread them in a thin layer to maximize aeration and allow them to dry in the shade (see Figure 2.1).
- Always maintain seeds in moisture-permeable containers such as cotton or paper bags, and ensure that air circulates freely between and through them.
- When conditions are hot and humid and collecting missions are long, further dry seeds using desiccants such as silica gel (the recommended ratio of seeds to silica gel is 3:2 to 1:1).
- Keep alternate layers of packed silica gel and packed seeds in a large airtight container to reduce seed moisture content.
- With small and many-seeded fleshy fruits (such as kiwi and strawberry), holding seeds inside fruits is the most practical option if collecting missions are short and logistics permit.
- Seed extraction should be avoided if fruits require after-ripening or if seeds are delicate or recalcitrant.



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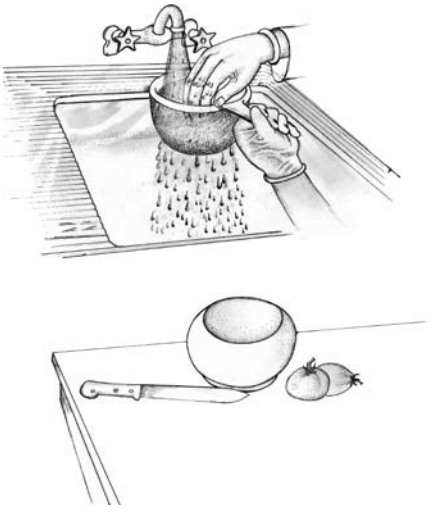

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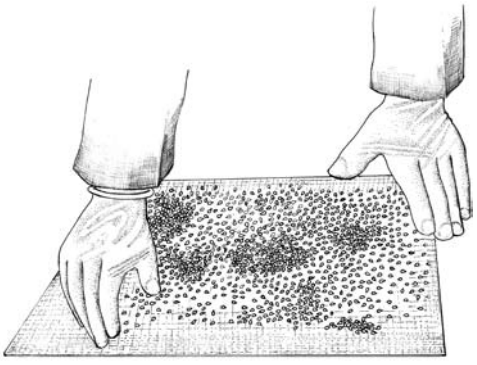

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Figure 2.1. Seed extraction from fleshy fruits.

Recruit couriers to accompany the team when collecting on long expeditions to remote areas and send perishable material or seeds with limited viability to the genebank as soon as possible.

Transporting the collected material to the genebank

The exploration team should ensure the safety of collected material until the time collecting ends and it is transported to the genebank. Exposing seeds to unfavourable environmental conditions during transport can be very damaging.

- Care must be taken to maintain the material at optimal temperature and safe moisture content even when transport distance is short.
- Ensure that the container holding seed samples is cushioned and that no damage is done to the seeds or fruits during transportation.

B. Germplasm acquisition by correspondence and exchange

Samples can be obtained by correspondence if it is known that collecting in the area of interest has already taken place. Genebanks may require documentation from countries or independent entities to certify that the consignment is free from genetically modified organisms (GMOs).

Identification of unique samples for acquisition

Maintaining samples in a genebank is expensive; genebanks should carefully check to ensure that samples do not already exist in their collections before acquisition. Since each genebank adopts its own numbering system, it is possible for the same accession to be recorded twice with different identification. Duplication in the collection is best identified by comparing relevant fields in the passport databases of donating and recipient genebanks.

Most errors are made during data entry, especially regarding spaces, hyphenation, case and spelling, which require careful checking when comparing databases to identify duplicate accessions.

Acquiring unique germplasm

Obtain complete passport information, including alternate names or identification numbers, pedigree and original source.

- Prepare a final list of unique accessions to be acquired.
- Send the final list of accessions identified for acquisition to the consignor to facilitate seed transfer.
- If material is being received from abroad, check the requirements of the host country's national phytosanitary authority and follow the procedures for seed import as described further below.

Germplasm accessions that have been screened and 'purified' through selection for desirable characteristics, and mutants identified in germplasm grow-outs, serve as important raw material for crop improvement. These include sources of resistance to biotic and abiotic constraints, male-sterile lines, dwarfs and other genetic stocks. Genebanks should acquire this material along with complete pedigree information.

Genebanks may also acquire elite germplasm generated in breeding programs for specific traits or with proven high yield. During acquisition, ensure that complete pedigree details and morphological data are included with the material.

Germplasm introduction and post-entry quarantine

Genebanks often acquire germplasm from areas where pests, pathogens and host species have co-evolved. The following classification will help genebank staff to assess the potential disease status of material to be acquired.

The risk of introducing new pests and pathogens is:

1. *low* for germplasm collected or produced in the area or country where the genebank is located;
2. *medium* for germplasm collected or produced in the same geographic region or continent where the host country is located; and
3. *high* for germplasm collected or produced in other continents and for vegetative material.

To reduce the risk of entry of pests, pathogens and weeds, some countries have legislation regulating the entry of exotic propagation material, including seeds. The importer must ensure compliance with all phytosanitary requirements of the destination country before importing any seeds.

General features of import regulations may include provisions such as the following:

- Consignments of plants and seeds may need to be imported through specific entry points, as determined by the national plant protection authority in the importing country.
- Seeds and planting material may need to be grown in isolation, or contained in a certified post-entry quarantine facility for a specified period of time, or to meet certain conditions.
- Additional provisions may be required for shipments consisting of plants or plant products.
- Importing soil, earth, sand, compost and plant debris accompanying seeds or planting material is usually prohibited.

Consignments will probably be inspected, and if necessary disinfected, by authorized phytosanitary officials before clearance, provided all other requirements of the importing country have been met. Failure to meet these requirements can cause unnecessary delays and may result in the consignment being destroyed.
Seed material requiring isolation may be planted under pest-proof glass, in screenhouses or in field plots. Phytosanitary officials carry

out periodic inspections during the growing period in which plants affected by seed-associated pests are destroyed while seeds collected from healthy plants are released to the genebank.

Procedure for seed import

At the planning stage, pay attention to the pests encountered on the target species and to the phytosanitary requirements for germplasm introduction.

1. Collect information on pests that are likely to be encountered in the country or area of seed collection or production.
2. Determine what plant parts these pests are found on.
3. Check the requirements for seed import with the national phytosanitary authority. If required, obtain a plant *import permit*[1] from the appropriate authority and send it to the consignor prior to import. All phytosanitary applications should be submitted to the national phytosanitary authority of the host country for approval.
4. If post-entry quarantine is required, grow each new accession under containment or isolation.
5. Plants should be observed periodically and those suspected of infection by seed-associated pests should be destroyed by incineration.
6. All symptom less plants should be tested for latent infections by viruses known to occur in the place of origin and in the country of maintenance; infected plants should be incinerated.
7. Seeds should be collected from healthy plants only.

Genetically modified organisms (GMOs)

Genebanks should be conscious of the dangers inherent in the inadvertent introduction of transgenes or genetically modified crops during germplasm assemblage, and must take measures to minimize these introductions (see Annex I and also section 5.3 of this manual). When planning for collecting or acquiring new accessions by other means, genebanks should conduct a risk analysis to determine:

1. whether transgenic events (commercial and research) in the relevant taxa are likely to be present in the area of collection or acquisition;
2. the distance between the collecting site and areas where transgenic events are situated; and
3. whether germplasm suppliers can provide adequate documentation of their management practices with respect to the material in question.

[1] An import permit is a written authorization by national plant protection services to import regulated items, including plants and plant products.

Further reading

Ebbels, D.L. 2003. Principles of plant health and quarantine. CAB International, Wallingford, UK.

FAO (2006). http://www.fao.org/waicent/FaoInfo/Agricult/AGP/AGPS/pgr/ITWG3rd/docsp1.htm

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International Treaty on Plant Genetic Resources for Food and Agriculture. FAO, Rome Italy. http://www.fao.org/ag/cgrfa/itpgr.htm. (Last visited: 11 October 2006)

Hay, F.R. and Smith, R.D. 2003. Seed maturity: when to collect seeds from wild plants. Pp. 97-133 in Seed conservation: Turning science into practice. (R.D. Smith, J.B. Dickie, S.H. Linington, H.W. Pritchard and R.J. Probert, eds.). Royal Botanic Gardens, Kew, UK.

2.2 Germplasm registration

What is registration?

Registration is the assignment of a unique identification number called an *accession number* for tracking each seed sample received by a genebank in order to distinguish it from other samples.

Why is it done?

Registration is carried out in order to allow genebanks to keep accurate records of samples and to produce inventory lists for conservation, distribution, and other aspects of germplasm management.

When is it done?

Registration is done when the sample first enters the genebank. For efficient management and use of the collections, register the samples if they meet the conditions described below.

How is it done?

Registration is carried out in several steps (see Flowchart 2.1).

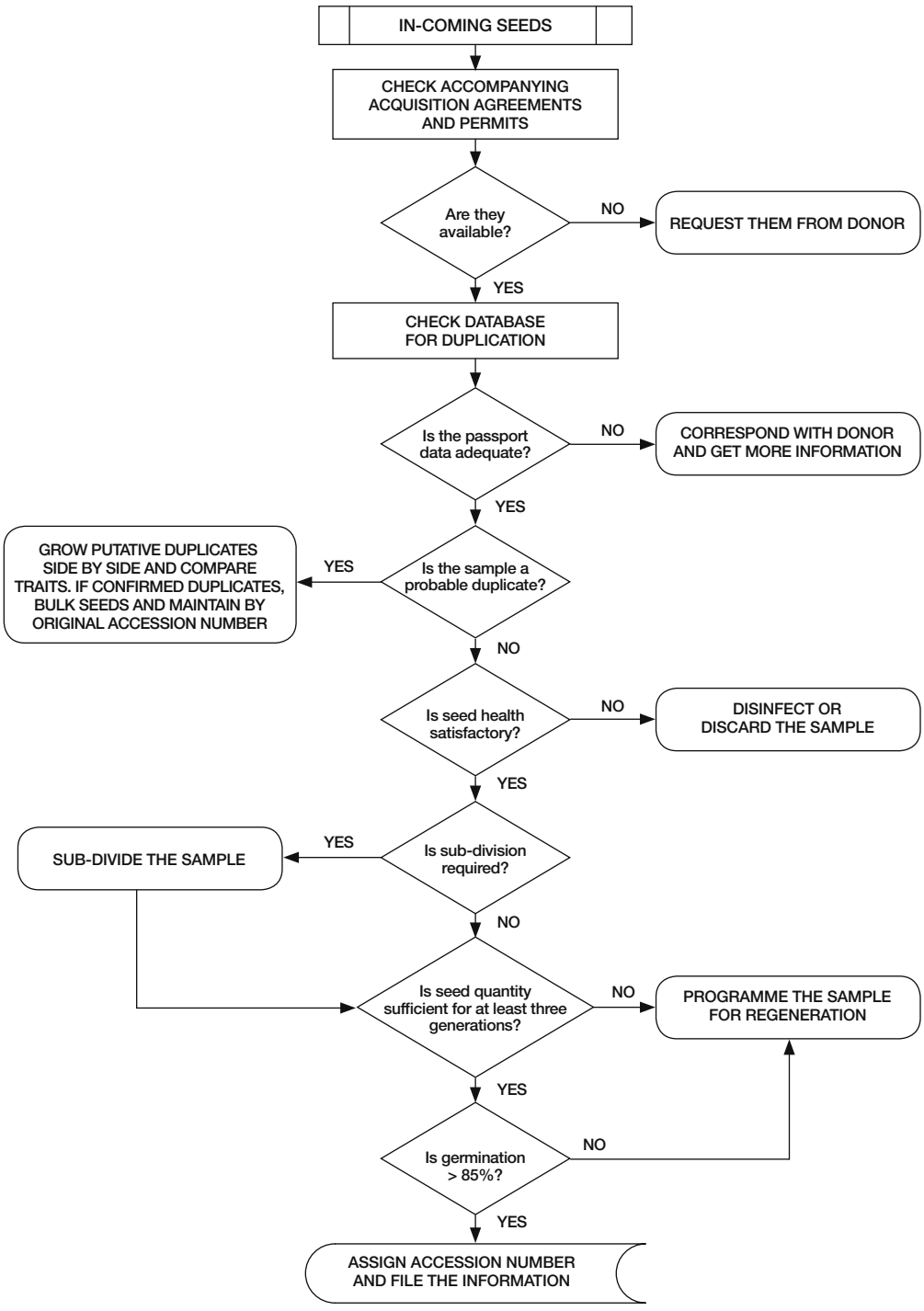
Step 1: Before registration

Prior to registration, the status of the samples should be verified to ensure that the following minimum conditions are met before acceptance in the genebank.

Acquisition agreements and permits

The samples should have been acquired from collectors, genebanks or other sources with appropriate material acquisition or transfer

Flowchart 2.1. Germplasm registration.



agreements and permits in line with national and international regulations regarding conservation, distribution and use (see Annex I for more information).

Passport information

Samples should be accompanied by adequate passport information, especially cultivar name, collector number and pedigree (for genetic stocks and improved material) to ensure that each sample does not already exist in the genebank. The minimum required passport data may include the following:

A. Samples from collecting missions:

- Common crop name and/or genus and species
- Collecting number
- Location of collecting site
- Country of origin
- Collecting date
- Phenology
- Collecting source
- Number of plants sampled

B. Samples received as donations:

- Common crop name and/or genus and species
- Accession name and/or other identification associated with the sample
- Pedigree information and breeding institute's details (for breeding lines)
- Phenology
- Acquisition source
- Country of origin
- Donor accession number (if applicable)

Distinctiveness

New samples should be genetically distinct from any other accessions already registered in the genebank. Two samples may have identical or very similar names and identical grain characteristics but may be genetically distinct, while samples with very different names may be genetically similar.

Morphological, biochemical and molecular approaches can be used to identify duplicates, depending on the facilities and resources available in the genebank. The following tests can be performed:

Morphological

- The suspected duplicates are grown side by side in the field or in a greenhouse and differences between morphological

characteristics such as plant height, flowering time, leaf and flower size, and shape and colour are compared.
- The candidate accession is defined as distinct when it is found to differ significantly in at least one characteristic from existing registered accessions.
- Morphology-based distinctness tests can be similar to the crop-specific set of characteristics that comply with guidelines set by the International Union for the Protection of New Varieties of Plants (UPOV, 1991). If necessary, these characteristics can be assessed over two or three seasons. This may not be practical in landraces with high within-accession variation, however.
- The statistical procedure to assess distinctness is the t-test.

Biochemical

When phenotypic comparison does not provide enough evidence of distinctness, biochemical methods such as electrophoresis of seed proteins and isozymes can be used for improving the comparison of morphological traits and to discriminate the samples.

Molecular

DNA markers such as AFLPs, SSRs and SNPs offer powerful discriminating tools and can be successfully applied in checking genetic relatedness between samples, provided that this approach is feasible and cost effective. For more details on molecular methods, see de Vicente and Fulton (2003).

If the samples being compared are confirmed to be duplicates, genebanks are recommended to bulk the seeds and treat them as one entity. If the sample is identical to an existing accession, maintain it under the original accession number.

Seed health

- Each sample should be accompanied by a *phytosanitary certificate* and additional declarations as required under the host country's phytosanitary regulations (see Chapter 7 for more details).
- Seed samples should be inspected by visual examination under a stereoscopic microscope. They should be free of pathogens, fungal growth, bacterial and viral infections, and insects.

Seed quality and quantity

Seeds should be of the highest quality and in adequate numbers for storage.

- In general, the percentage germinated should not be below 85% for cultivated species or below 75% for the wild species (for more information on germination testing, see Chapter 5).

Box 2.1. Base unit for registration.

The minimum number of seeds for registration (*base unit*) can be estimated from the standard sample size used for regeneration and the sample viability according to the following equation:

Number of seeds required for registration = Desired plant population for regeneration x minimum number of regenerations / (Germination % × Expected field establishment 1∗%)[†]

Example:

Desired plant population for each regeneration = 100
Germination = 95%
Expected field establishment = 90%
Minimum number of regenerations (safety factor) = 3

Base unit or minimum number of seeds for registration = $\frac{(100 \times 3)}{(0.95 \times 0.90)}$ = 351 seeds

[†] Germination and field establishment are expressed in decimals; for example, 95% is expressed as 0.95. Plant establishment is generally 5% less than the germination percentage in poor conditions and 1% less in good conditions.

- Seed quantity should be sufficient to conduct at least three regenerations. This will ensure that seeds are still available for another planting even if the first attempt to regenerate fails (see Box 2.1).

What if minimum conditions are not met?

If the sample does not meet the required conditions, assign a *temporary number* until the sample is ready to receive a permanent registration number. The temporary number should be easily distinguishable from other accession numbers.

Agreements and permits

Contact the collector or donor for the necessary agreements defining the status of samples with regard to conservation and further use.

Duplicate accessions

Confirm duplication and assign the seeds as a new seed lot under the original accession number.

Missing passport information

Write to the collector or donor of germplasm to request missing information.

Poor seed health

If seeds contain pathogens or insects, send the sample to a phytopathologist or entomologist for treatment. If it is possible to acquire a replacement sample, immediately incinerate the sample and make note of the action taken and the justification; request a fresh sample from the donor.

Inadequate seed quality and quantity

Regenerate the sample immediately.

If samples are registered without adequate passport data, their identities and biological status will remain unknown, hampering their use. Failure to regenerate samples with low viability or very few seeds may result in loss of the accession, leaving gaps in inventory.

Restructuring samples

In self-pollinating crops, if a sample comprises of a physical mixture of two or more distinct lines or species, they may be subdivided and maintained as distinct accessions. In this case, subdividing the sample into its components helps in effective maintenance of genetic integrity. *Note that subdivision should not be undertaken if variation in the original sample is continuous, as in highly cross-pollinating crops.*

Step 2: Procedure for registration

If the sample meets the minimum conditions described above, it may be accepted for registration and assigned an accession number using the following procedure:

1. Arrange the material in alphabetical order by variety name or in numerical order by collection number, depending on the identification provided.
2. Check all packets against the list accompanying the samples.
3. If no list is provided or seeds do not correspond to the data, prepare a new list. Check again to confirm that all packets have been included.
4. Check the passport data file to determine the last accession number given.
5. Assign the next ascending accession number to the first sample on the list and consecutive numbers to succeeding samples.
6. Write the accession number clearly on the packet using a permanent marker and on the list of new samples.
7. Enter the details in the passport data files of the genebank's documentation system. For each accession, record all passport data, original identification data and registration date in the designated fields of the passport data file.
8. If data are missing, leave the field blank and contact the donor to supply the missing data.

Numbering procedures for new genebanks

A genebank numbering system should be simple and practical to use.

- Use a strictly numeric system that is sequential (1, 2, 3). Assigned numbers are usually preceded by an acronym (such as GBK for Genebank of Kenya) to identify each sample with its registered genebank. Additional information such as year of acquisition and crop code should not be incorporated into an accession number.
- If large collections of germplasm are maintained, separate but sequential accession numbering may be given for each crop. This approach is not recommended if the genebank is small or has many crops, however.

- Avoid assigning 'reserved' numbers for particular crops (for instance, 1 to 500 for maize, 501 to 1000 for cowpea) or for wild species when using a single numbering system.

Documentation

Documenting the information received along with a sample is an important aspect of registration. Information documented at registration consists of passport data providing basic information for identification and general management of individual accessions.

Much of this information is either recorded when the sample is collected or accompanies the sample if it is received from other sources. The use of internationally accepted descriptor lists to document passport information simplifies data exchange between genebanks. The standard Multi-crop Passport Descriptor (MCPD) list developed by FAO and IPGRI is available at www.bioversityinternational.org/publications/pdf/124.pdf.

Further reading

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de Vicente, C. and Fulton, T. 2003. Using molecular marker technology in studies on plant genetic diversity: Learning module Vol 1. IPGRI, Rome, Italy.

International Union for the Protection of New Plant Varieties (UPOV). 1991. International Convention for the Protection of New Varieties of Plants. UPOV, Geneva. (http://www.upov.int)

3. Cleaning

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

3. SEED CLEANING

What is seed cleaning?

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

Why clean seeds?

Seed cleaning is necessary to:

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

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

When to clean seeds

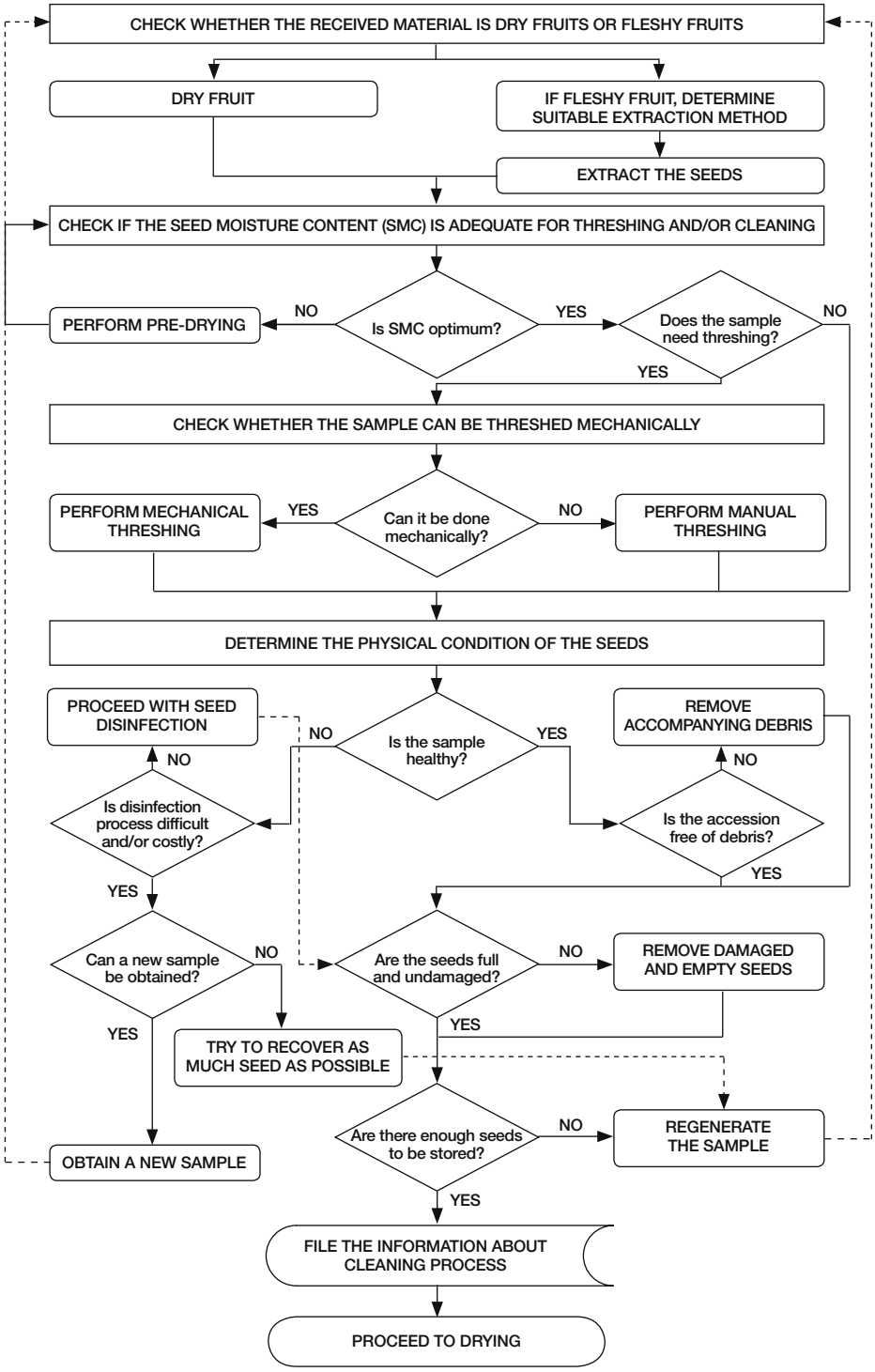
Seeds should be cleaned immediately after harvest or soon after they arrive at the genebank. Fruits may be soft and fleshy like drupes with fleshy pulp or hard and leathery like pods. Seed extraction is therefore the first step in seed cleaning.

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

Extracting seeds from fruits

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

Flowchart 3.1. Seed cleaning.



Extraction of seeds from dry dehiscent fruits

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

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

Seeds can be threshed either by hand or mechanically.

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

Extraction of seeds from dry indehiscent fruits

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

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

Sweating technique for forage grasses

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

Extraction of seeds from fleshy fruits

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

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

Mucilaginous seeds

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

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

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

Fruits with pulp firmly attached

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

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

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

Stone fruits

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

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

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

How to clean seeds

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

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

Step 1: Separation from debris

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

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

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

Step 2: Examining seeds for insect and fungal damage

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

Step 3: Examining seeds for mechanical damage and empty seeds

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

Step 4: Purity analysis

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

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

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

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

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

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

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

Example:

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

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

Step 5: Verification

After cleaning:

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

Useful equipment

The following equipment is useful for seed cleaning:

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

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

Documentation

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

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

Further reading

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

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

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

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

4. SEED MOISTURE CONTENT DETERMINATION AND DRYING

4.1 Seed moisture content determination

What is seed moisture content?

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

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

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

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

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

Why is it important to determine seed moisture content?

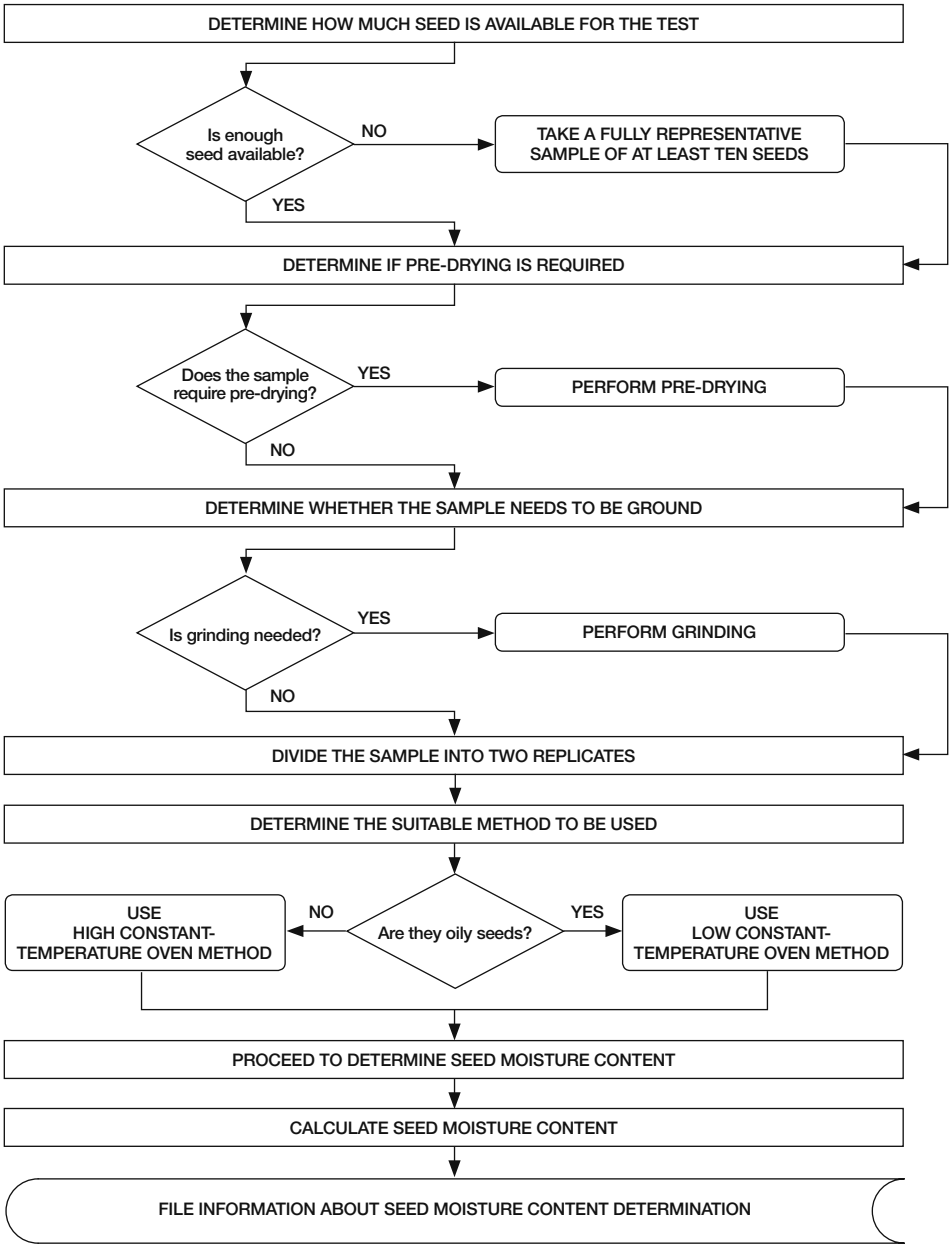
Moisture content is the most important factor determining the rate at which seeds deteriorate and has profound impacts on storage longevity of seeds in genebanks. Even small changes in moisture content have large effects on storage life. It is important to determine moisture content before storage in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

Determining seed moisture content

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

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

Flowchart 4.1. Seed moisture content determination.



Oven-drying method

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

ISTA (2005) has prescribed two different oven-drying methods for determining moisture content, based on the chemical composition of seeds:

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

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

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

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

*grinding required.

Pre-drying

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

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

Equipment

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

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

Sample size and sampling

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

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

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

Grinding

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

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

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

Moisture content determination

High constant temperature method for non-oily seeds

Moisture content is determined in the following way:

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

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

   where,

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

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

Table 4.3. Recording and calculation of seed moisture content.

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

Example:

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

Calculation:

Rep 1:

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

Rep 2:

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

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

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

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

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

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

Low constant temperature method for oily seeds

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

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

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

Moisture balances

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

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

Non-destructive methods for moisture determination

Quick moisture meters

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

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

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

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

Calibration of quick moisture meters

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

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

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

Digital humidity sensors

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

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

Further reading

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

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

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

4.2 Seed drying

What is seed drying?

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

Why are seeds dried?

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

When are seeds dried?

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

To what moisture content should seeds be dried?

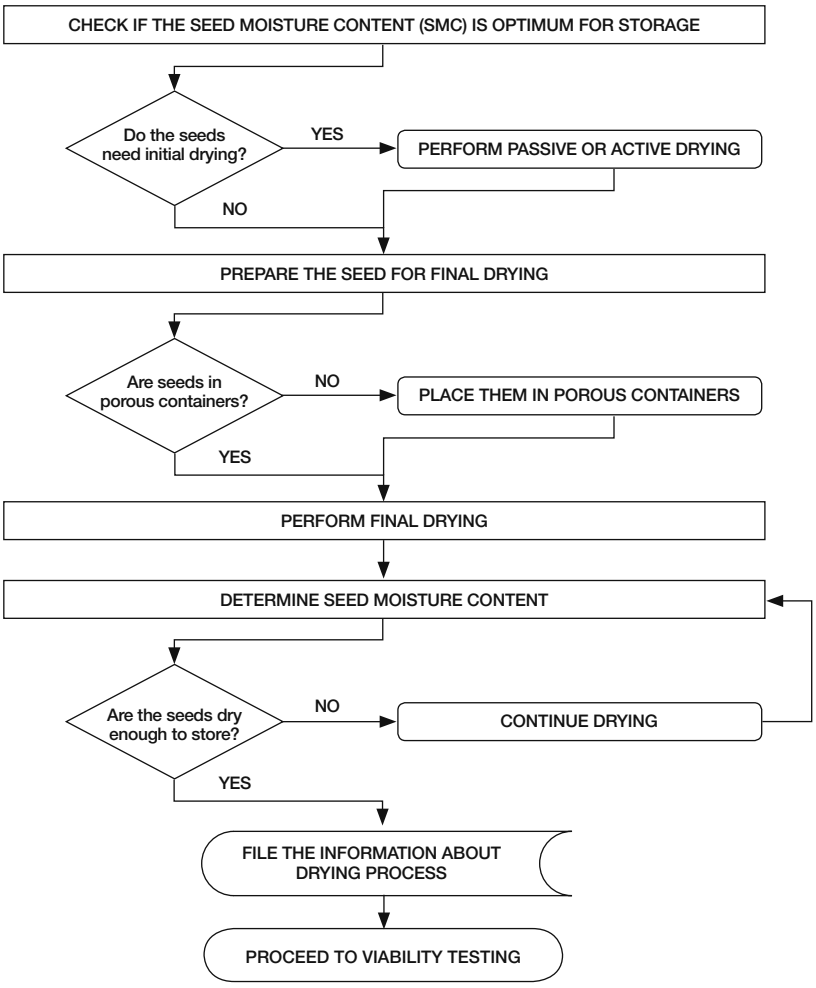
The optimal moisture content for storage depends on the species and the intended period of storage. It is important to adopt an appropriate drying regime, in which the relative humidity and temperature of the drying air are regulated to achieve the target moisture content.

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

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

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

Flowchart 4.2. Seed drying.



Critical moisture content

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

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

Principles of seed drying

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

Equilibrium moisture content and moisture isotherms

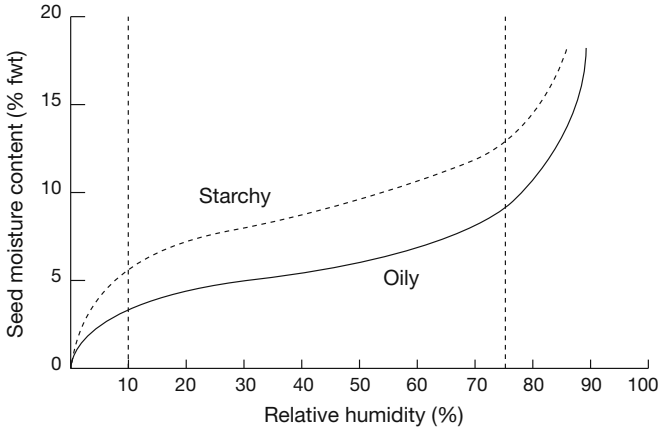
The water content of seeds at equilibrium with the relative humidity of the surrounding air is referred to as equilibrium moisture content. Understanding the relationship between equilibrium seed moisture content and relative humidity is important in determining the appropriate drying regime for seeds.

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

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

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

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



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

Figure 4.1. Moisture isotherms.

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

How to prepare moisture isotherms

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

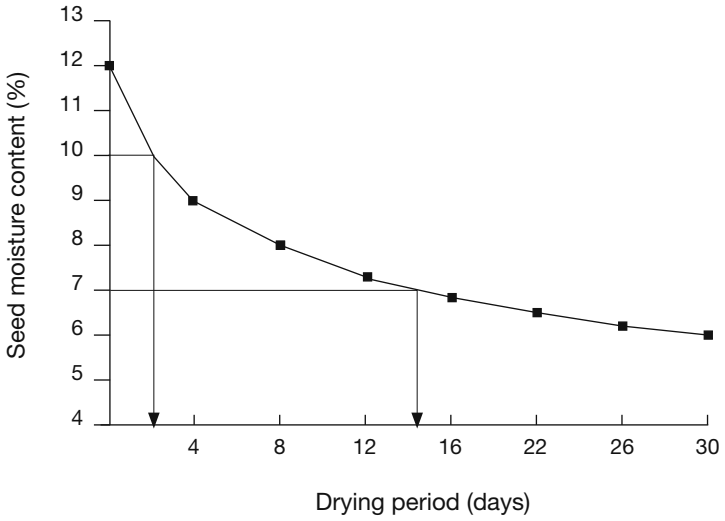
Salt	Corresponding RH (%)
Sodium hydroxide	7.5
Lithium chloride	13
Magnesium chloride	32
Magnesium nitrate	54
Ammonium nitrate	65
Sodium chloride	75
Potassium chloride	85

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

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

Assessing desiccation sensitivity

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



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

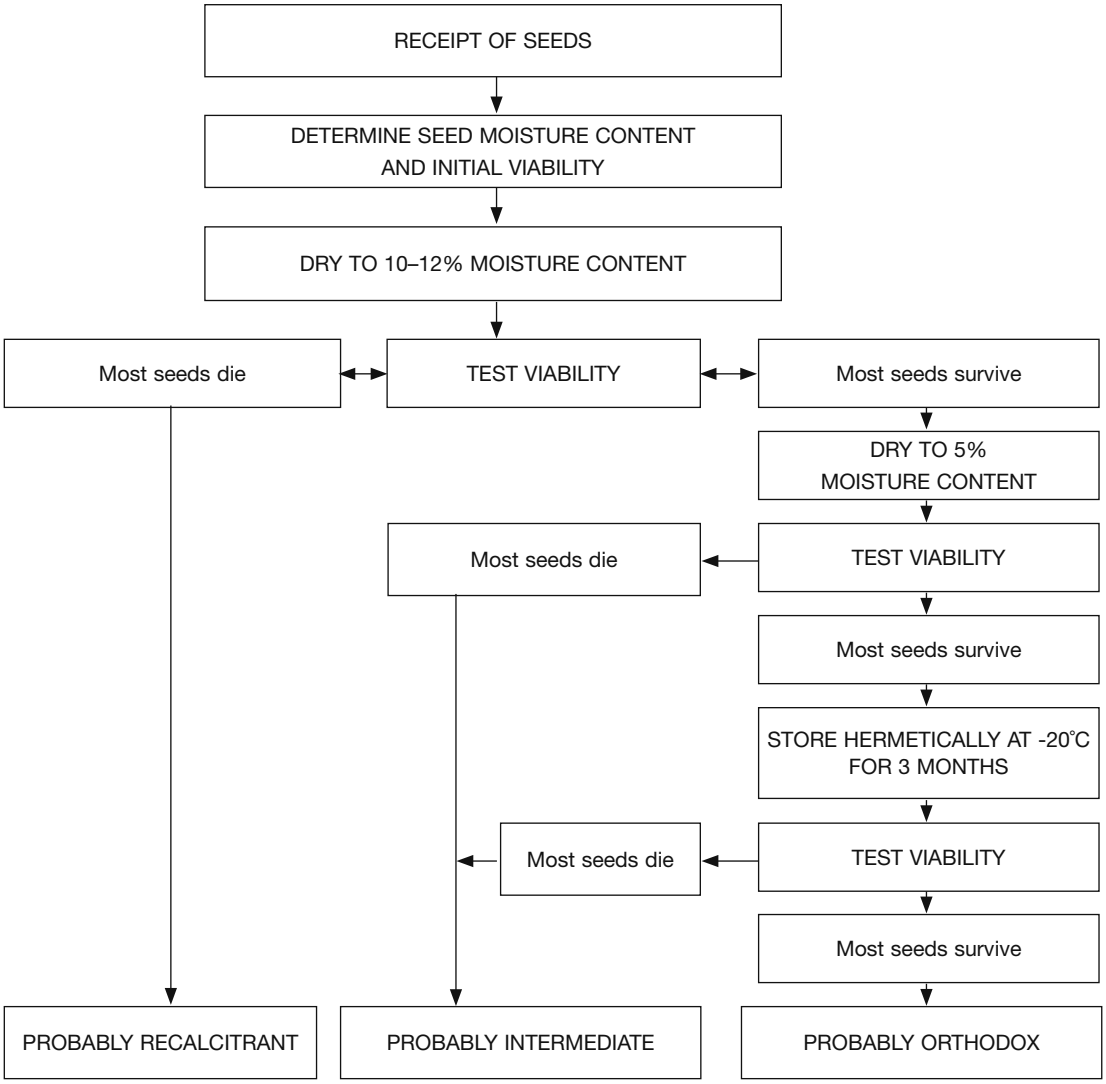
Figure 4.2. Predicting drying time.

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

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

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

Flowchart 4.3. Protocol to determine seed storage behaviour.



Source: Hong and Ellis (1996).

Seed-drying procedures

Step 1: Predict moisture content and drying period

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

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

Prediction of drying time

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

Prediction of drying time by weight loss

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

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

Example:

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

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

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

Prediction of drying period from mean drying curves

In general, seeds dry at an exponential rate until equilibrium moisture content is reached. The rate of drying of different seed lots of the same species will be more or less equal under the same environmental conditions. Drying curves can therefore be used for predicting the

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

How to prepare mean drying curves

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

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

Using mean drying curves to predict drying time

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

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

Step 2: Prepare seeds for drying

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

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

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

Step 3: Dry the seeds

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

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

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

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

Box 4.1. Options for initial drying.

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

Dehumidified drying

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

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

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

Silica gel-drying

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

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

Calcium chloride drying

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

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

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

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

Saturated salt solutions

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

To prepare the salt mixture:

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

Other low-cost methods

Self-defrosting refrigerator

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

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

Shade-drying

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

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

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

Documentation

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

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

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

Further reading

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Storage of seeds with high initial quality will maximize accession longevity. Monitoring viability during storage facilitates timely identification of accessions that require regeneration to ensure continued availability of conserved germplasm.

5. SEED QUALITY TESTING

5.1 Seed viability testing

What is seed viability?

Seed viability is the measure of how many seeds in a lot are alive and could develop into plants that will reproduce under appropriate field conditions.

Why should seed viability be determined?

It is very important that seeds stored in the genebank are capable of producing plants when sown in the field. They must have high viability at the start of storage and maintain it during storage. Seeds with a high initial viability will also survive longer in storage. Seed viability declines slowly at first and then rapidly as seeds age. It is important to know when this decline occurs in order to take action to regenerate the accession. Excessive deterioration will lead to loss of material.

When should viability be determined?

Viability of accessions should be determined:

- before seeds are packaged and placed in the genebank; and
- at regular intervals during storage.

Viability-testing can take from a few days to weeks, depending on the species. If possible, the results of viability tests should be made available before seeds are packaged and placed in the genebank so that poor-quality seeds can be identified and regenerated.

While awaiting the results of viability tests or if there is a delay in conducting the viability tests before storage, seeds should be placed in a cool environment to minimize their deterioration.

How should viability be determined?

Many different methods are available to test seed viability. The most accurate and reliable method is the germination test. There are also biochemical tests, which have the advantage of being quicker, but are not as accurate as the germination test.

They also require special skills to conduct and interpret. These tests are not usually recommended for general use to test for seed viability in genebanks.

What is a germination test?

A germination test is performed to determine what proportion of seeds in an accession will germinate under favourable conditions and produce normal seedlings (seedlings that have the essential structures—roots, shoots and sufficient food reserves) capable of development into reproductively mature plants (see Flowchart 5.1).

How is germination tested?

Basic requirements for seed germination are: water, oxygen, light and suitable temperature. Seeds of different species have different requirements and no general set of conditions can be relied upon to germinate seeds of all species. Seeds of some species are more tolerant and germinate in a wide range of conditions but complete germination can only be achieved under optimum conditions.

How many seeds should be tested?

- A fixed-sample size germination test using 200 seeds is recommended to determine viability at the beginning of storage.
- The International Standards for Genebanks (FAO/IPGRI, 1994) recommend using a minimum of two replicates with 100 seeds per replicate. If the test results show that germination is below 90%, an additional 200 seeds should be tested using the same method. Overall seed viability is taken as the mean of the two tests.

For species with large seeds that have a low seed-multiplication rate and those with problems in seed regeneration (such as wild species), it may become difficult to expend 200 seeds for a germination tests. In this case, two replicates of 50 or 25 seeds each can be used, depending on the available quantity.

When has a seed germinated?

Seed germination can be defined as the resumption of growth of the embryo and emergence or protrusion of the radicle from the covering structures. For seed testing in genebanks, germination is not complete until the seedling can be judged as normal according to specific criteria for each species (see Association of Seed Analysts [AOSA], 2005; ISTA, 2005). This is because the intent of seed testing is to give an indication of how the seeds will perform as propagules in the field.

Germination testing

Step 1: Preparation for germination testing

1. Check the specific requirements for temperature, light and any other treatments needed to test germination of a species (see Table 5.1).
2. Check that the equipment and appropriate environment are available to fulfil these requirements. If not, the best possible alternatives should be found.

Flowchart 5.1. Germination testing.

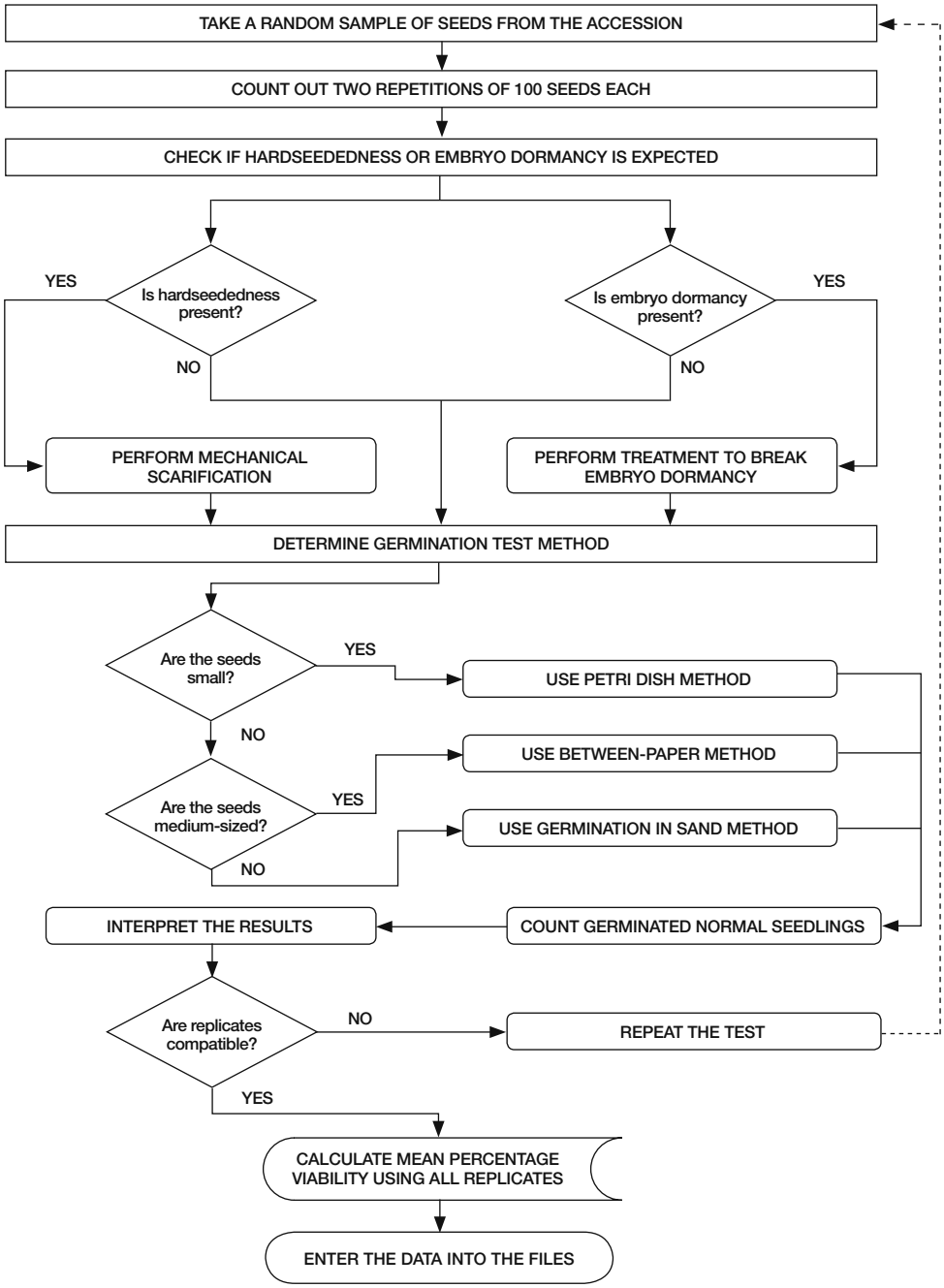


Table 5.1. Guidelines for testing germination of the most common crop species. Refer to ISTA (2005) or AOSA (2005) for information on other crops.

Crop	Species	Substrate*	Temp (°C)**	First, Final count (days)	Special treatments; Additional directions for fresh and dormant seeds
Alfalfa	*Medicago sativa*	TP; BP	20	4, 7	Mechanical scarification of hard seeds
Annual rape	*Brassica napus*	BP, TP	20/30	3, 7	
Barley	*Hordeum vulgare*	BP; S	20	4, 7	Pre-chill at 5°C or 10°C for five days
Bermuda grass	*Cynodon dactylon*	TP	20/30	7, 21	Light; KNO_3
Berseem	*Trifolium alexandrinum*	TP; BP	20	3, 7	
Black gram	*Vigna mungo*	BP	20/30; 25; 20	3, 7	
Black mustard	*Brassica nigra*	TP; BP	20/30; 20	3, 7	Light; KNO_3 and pre-chill at 10°C for three days
Bottle gourd	*Lagenaria siceraria*	BP; S	20/30; 20	14	
Buck wheat	*Fagopyrum esculentum*	BP; TP	20/30; 20	3, 6	
Cabbage	*Brassica oleracea* var. *capitata*	TP; BP	20/30; 20	3, 10	Pre-chill at 5° or 10°C for three days; KNO_3 and light
Carrot	*Daucus carota*	TP; BP	20/30; 20	6, 14	GA_3 50 ppm
Castor bean	*Ricinus communis*	BP; S	20/30	7, 14	
Cauliflower	*Brassica oleracea* var. *botrytis*	TP; BP	20/30; 20	3, 10	Pre-chill at 5° or 10°C for three days; KNO_3 and light
Chickpea	*Cicer arietinum*	BP	20	5, 8	Mechanical scarification of hard seeds
Chicory	*Cichorium intybus*	TP	20; 20/30	5, 14	Light; KNO_3
Coriander	*Coriandrum sativum*	TP; BP	15	6, 21	
Cotton	*Gossypium* spp.	BP; S	20/30; 25	4, 12	De-linting; Mechanical scarification of hard seeds
Cowpea	*Vigna unguiculata*	BP; S	20/30; 25	5, 8	
Cucumber	*Cucumis sativus*	TP; BP	20/30	3, 7	Keep substrate on dry side
Eggplant	*Solanum melongena*	TP; BP; S	20/30	7, 14	Light; KNO_3
Faba bean	*Vicia faba*	BP; S	20	4, 14	Pre-chill at 10°C for three days
Field bean	*Phaseolus vulgaris*	BP; S	20/30; 25; 20	5, 8	

Crop	Species	Substrate*	Temp (°C)**	First, Final count (days)	Special treatments; Additional directions for fresh and dormant seeds
Finger millet	*Eleusine corocana*	TP	20/30	8	KNO_3
Flax	*Linum usitatissimum*	BP; TP	20/30; 20	3, 7	
Foxtail millet	*Setaria italica*	TP	20/30	4, 10	
Grain Amaranth	*Amaranthus* spp.	TP	20/30; 20	7, 14	
Grass pea	*Lathyrus sativus*	BP; S	20	4, 14	Mechanical scarification of hard seeds
Hot pepper	*Capsicum frutescens*	TP; BP	20/30	6, 14	Light and KNO_3
Indian mustard	*Brassica juncea*	TP; BP	20/30	3, 7	Light; Pre-chill at 10°C for seven days and test for five additional days
Lentil	*Lens culinaris*	BP, S	20	5, 10	Mechanical scarification of hard seeds
Lettuce	*Latuca sativa*	TP; BP	20	7	Light; pre-chill
Lima bean	*Phaseolus lunatus*	BP; S	20/30; 25	5, 9	
Lupin	*Lupinus angustifolius; L. albus*	BP; S	20	3, 10	
Maize	*Zea mays*	BP; S	20/30; 25; 20	4, 7	
Mung bean	*Vigna radiata*	BP; S	20/30; 25	3, 7	
Muskmelon	*Cucumis melo*	BP; S	20/30	4, 10	Keep substrate on dry side
Oat	*Avena sativa*	BP; S	20	5, 10	Pre-chill at 5°C or 10°C for five days and test for ten days
Okra	*Abelmoschus esculentus*	BP; TP	20/30	4, 14	
Onion	*Allium cepa*	BP; TP	20	6, 10	
Orchardgrass	*Dactylis glomerata*	TP	15/25	7, 21	Light; Pre-chill at 5°C or 10°C for seven days
Pea	*Pisum sativum*	BP; S	20	8	
Peanut	*Arachis hypogaea*	BP; S	20/30; 25	5, 10	Ethephon, 0.2%
Pearl millet	*Pennisetum glaucum*	TP; BP	20/30; 25	3, 7	
Pigeonpea	*Cajanus cajan*	BP	25	5, 10	Mechanical scarification of hard seeds

Crop	Species	Substrate*	Temp (°C)**	First, Final count (days)	Special treatments; Additional directions for fresh and dormant seeds
Potato	*Solanum tuberosum*	TP; BP	20/30; 20	8, 16	GA_3, 2000 ppm
Pumpkin	*Cucurbita maxima*	BP; S	20/30; 25	4, 7	Keep substrate on dry side
Radish	*Raphanus sativus*	TP; BP	20/30; 20	4, 6	
Red clover	*Trifolium pratense*	TP; BP	20	4, 10	Pre-chill at 5°C or 10°C for five days
Rice	*Oryza sativa*	TP; BP; S	20/30; 25	5, 14	Preheat at 40°C for five days
Rye	*Secale cereale*	TP; BP; S	20	4, 7	Pre-chill at 5°C or 10°C for five days
Rye grass	*Lolium perenne*	TP	15/25; 20	5, 14	KNO_3 and pre-chill at 5°C or 10°C for five days
Safflower	*Carthamus tinctorius*	TP; BP	20; 25;	4, 14	Light at 15°C
Sesame	*Sesamum indicum*	TP	20/30	3, 6	
Sorghum	*Sorghum bicolor*	TP; BP	20/30; 25	3, 10	Pre-chill at 5°C or 10°C for five days
Soya bean	*Glycine max*	BP; S	20/30; 25	5, 8	
Squash	*Cucurbita pepo; C. moschata*	BP; S	20/30	4, 7	Keep substrate on dry side
Strawberry	*Fragaria ananassa*	TP	20/30; 20	28	Light
Sugar beet	*Beta vulgaris* subsp. *vulgaris*	TP, BP, S	20/30; 20	3, 10	Pre-wash and dry at a maximum of 25°C
Sunflower	*Helianthus annuus*	BP; S	20/30; 25; 20	3, 7	
Sweet clover	*Melilotus albus*	TP; BP	20	4, 7	
Timothy grass	*Phleum pratensis*	TP	20/30	5, 10	Light; KNO_3 and Pre-chill at 5°C or 10°C for five days
Tobacco	*Nicotiana tabacum*	TP	20/30	4, 14	Light
Tomato	*Lycopersicon esculentum*	TP; BP	20/30	5, 14	Light; KNO_3
Triticale	*Triticosecale*	TP; BP; S	20	4, 7	Pre-chill at 5°C or 10°C for five days
Vetch	*Vicia sativa*	BP; S	20	5, 10	
Watermelon	*Citrullus lanatus*	BP; S	20/30; 25	4, 14	Keep substrate on dry side; Test at 30°C
Wheat	*Triticum aestivum*	TP; BP; S	20	4, 7	Pre-heat (30°–35°C); Pre-chill; GA_3
White clover	*Trifolium repens*	TP; S	20	3, 10	Pre-chill at 5°C or 10°C for five days

*TP = Top of paper, BP = Between paper, S = Sand
**20/30 = alternating temperatures of 20°C applied for eight hours per day and 30°C for 16 hours.

3. Take a random sample of seeds from each accession after gently mixing the seed lot in its container or by spreading them on a clean surface and mixing thoroughly.
4. Count out 200 seeds (or fewer, depending on availability) for each test. Return excess seeds back to the container.
5. Divide these seeds into at least two replicates.
6. If the seeds are very dry (with moisture content below 8%) and are likely to suffer from imbibition damage, it may be necessary to raise the moisture content (this process is called *humidification*) to 15–17% before testing for germination (see Box 5.1).

Box 5.1. Humidification of dry seeds.

Small seeds

1. Spread the seeds uniformly on a petri dish.
2. Place three very moist paper towels flat inside a large polythene box.
3. Place the petri dishes (without covers) containing seeds on top of the moist paper and close the box with a tight-fitting lid.
4. Place the box in an incubator at 20°C for 24 hours or more, depending on the initial moisture content.

Large seeds

1. Place the seeds in porous bags made of mosquito netting or similar material.
2. Place the bags on top of a gauge above water in a desiccator. Care should be taken to avoid direct contact of seeds with water.
3. Place the desiccator at 20°C for about 48 hours. The seed layer should not be more than one seed deep to enable all seeds to absorb moisture equally from the atmosphere.

Seeds of wild species with hard seed coats may require scarification (puncture the seed coat with a razor blade, sandpaper or scalpel without damaging the embryo) before humidification or sowing.

Step 2: Setting up the germination test

Although several methods are available to test germination, the four methods described below are suggested; they can be used for most species and give uniform results:

1. Top-of-paper method
2. Between-paper method
3. Germination in sand
4. Agar method

Absorbent paper is used as substrate for germination in the first two methods.

All new batches of paper substrate should be tested for quality upon receipt.

Paper substrate quality

It is important that high-quality paper be used as a substrate in order to obtain uniform germination and reproducible results. If possible, the paper should meet the following specifications (ISTA, 2005):

- The paper used as substrate[10] should not be toxic to developing seedlings.
- It should be able to absorb and supply sufficient moisture for the seeds to germinate.
- It should be strong enough not to disintegrate when handled, and not to be penetrated by the roots of developing seedlings.
- It should have a neutral pH of 6–7.

Simple test for paper quality

A. Presence of toxic substances

1. Cut the paper to size and place in a 9–cm Petri dish.
2. Moisten the paper with sufficient water.
3. Test using seeds of sensitive species like Bermuda grass (*Cynodon dactylon*), petunia (*Petunia hybrida)* or tobacco (*Nicotiana tabacum*) to observe germination on the moistened paper.
4. Evaluate root development after five days.
   - Symptoms of paper toxicity include shortened and discoloured root tips.

B. Paper strength

1. Moisten the paper and hold it in the air from one corner.
   - The paper should not fall apart.

C. Moisture absorption

1. Cut the paper into 10-mm wide strips.
2. Hold vertically with about 20 mm of the paper immersed in water.
3. Measure the height above the level that the moisture has risen to.
   - The minimum standard is a 30 mm rise in two minutes.

Controlling fungi in germination tests

Fungal contamination is a common occurrence during germination testing, especially with legume seeds; it is usually associated with immature, damaged or old seeds. It can also arise during pre-treatments like seed extraction or as a result of hygiene problems in the seed-testing area. Adopt the following laboratory practices to minimize the risk of fungal contamination:

1. Clean and disinfect (by surface sterilization with 70–95% alcohol or 20% domestic bleach) the test area and incubators between batches to limit the spread of fungal attack; washing hands, benches and inside incubators with hot soapy water is a simple but effective technique to reduce contamination.

[10] Examples of standard substrates include grade 181 filter paper from Whatman and 400PT non-toxic paper towels from Seedburo.

2. Space seeds properly and ensure that individual seeds do not touch each other. Use greater numbers of replicates, if necessary.
3. Provide an optimum environment for germination so that seeds germinate quickly—the temperature regime should be suitable and the test environment must be well-aerated.
4. Ensure cleanliness of germination test media and containers—make sure that they are not sources of inoculum. Sterilize container surfaces by wiping them with 70–95% alcohol or soaking them in 20% bleach or hot water at 55°C for 10–15 minutes.
5. Avoid imbibition injury (by prior humidification of the seeds) that could lead to leakage of cell contents, which provide sources of nutrients to fungi.
6. Promptly remove decaying seeds to prevent the spread of fungi to neighbouring seeds. If contamination is increasing, wash the seeds well in 1–10% bleach and re-commence the test in a clean container on a new substrate.
7. Remove seed-covering structures (such as glumes) before tests when they are found to be sources of infection.
8. Remove seeds that have germinated before harvest and subsequently dried, as they can be sources of infection.

While these practices minimize the risk of fungal contamination, dressing seeds with fungicides such as Thiram or Benlate, or sterilizing surfaces with sodium hypochlorite reduces fungal attack during germination testing. Use of fungicides may affect the results of germination tests and may constitute a health hazard to seed analysts, however. They should be used only when essential, but are extremely useful during field sowing for regeneration.

Seed dressing

1. Add a pinch of fungicide to the container holding seeds prepared for germination testing.
2. Thoroughly shake the container so that seeds receive a uniform coating of fungicide.

Surface sterilization

1. Soak seeds for ten minutes in a 1% solution of sodium hypochlorite. The concentration of household bleach is usually 5% sodium hypochlorite. Add 80 ml of distilled water to 20 ml of bleach to get a 1% solution.
2. Rinse the seeds thoroughly before testing for germination.

Top-of-paper method

This method is most suitable for species with seeds smaller than 2 mm in diameter such as small-seeded vegetables and forage grasses. The seeds are germinated on top of moist absorbent paper in containers

with close-fitting lids to prevent moisture loss. Commonly used containers include 9 cm glass or plastic Petri dishes.

1. Sterilize container surfaces by wiping with 70–95% alcohol or soaking in 20% bleach or hot water at 55°C for 10–15 minutes.
2. Cut the absorbent paper to the size and shape of the container. For Petri dishes, round filter paper such as Whatmann Grade 181 of appropriate diameter can be used.
3. Place the paper substrate at the bottom of the container or Petri dish.
4. Label containers with accession number, number of replicate and testing date; use a pencil or permanent marker for labelling.
5. Add the required volume of distilled water. If distilled water is not available, boiled and cooled tap water can be used. The volume of distilled water depends on the thickness of the paper substrate and the size of container. For Whatman Grade 181 filter paper in 9 cm Petri dishes, 4 ml of water is required. The filter paper should not be so wet that a film of water forms around the finger when it is pressed.
6. Firm down the paper substrate in the container using an upside-down funnel or tweezers.
7. Spread the seeds uniformly on the surface of the paper so that they are not touching. It is recommended that the distance between seeds should be at least three to five times the seed diameter.
8. Cover the containers and ensure that there is no air lock resulting from excess moisture on the covers.
9. Place the containers in a germinator or incubator maintained at the recommended temperature for germination of the species (see Table 5.1).
10. Check the moisture level of the substrate regularly, especially when humidity inside the cabinets is not controlled or when the temperature is set at 25°–30°C. Blotters usually need to be watered several times during the test. Alternatively, keep the containers in a thin plastic bag (loosely folded at the open end, but not sealed to allow diffusion of oxygen) to prevent the substrate from drying.
11. Run the test for the recommended period (see Table 5.1) and count the number of seeds that have germinated.
12. If some seeds have not germinated and appear to be dormant, treat with appropriate techniques to stimulate germination (see Table 5.1) and continue the test until all seeds have germinated or until no further germination has occurred after two consecutive counts.
13. Make a note of the seeds that did not germinate but are firm and sound at the end of the first count, and those that failed to germinate and are presumed dead at the end of the germination test.

Figure 5.1 shows the stages in testing germination using blotters in Petri dishes.

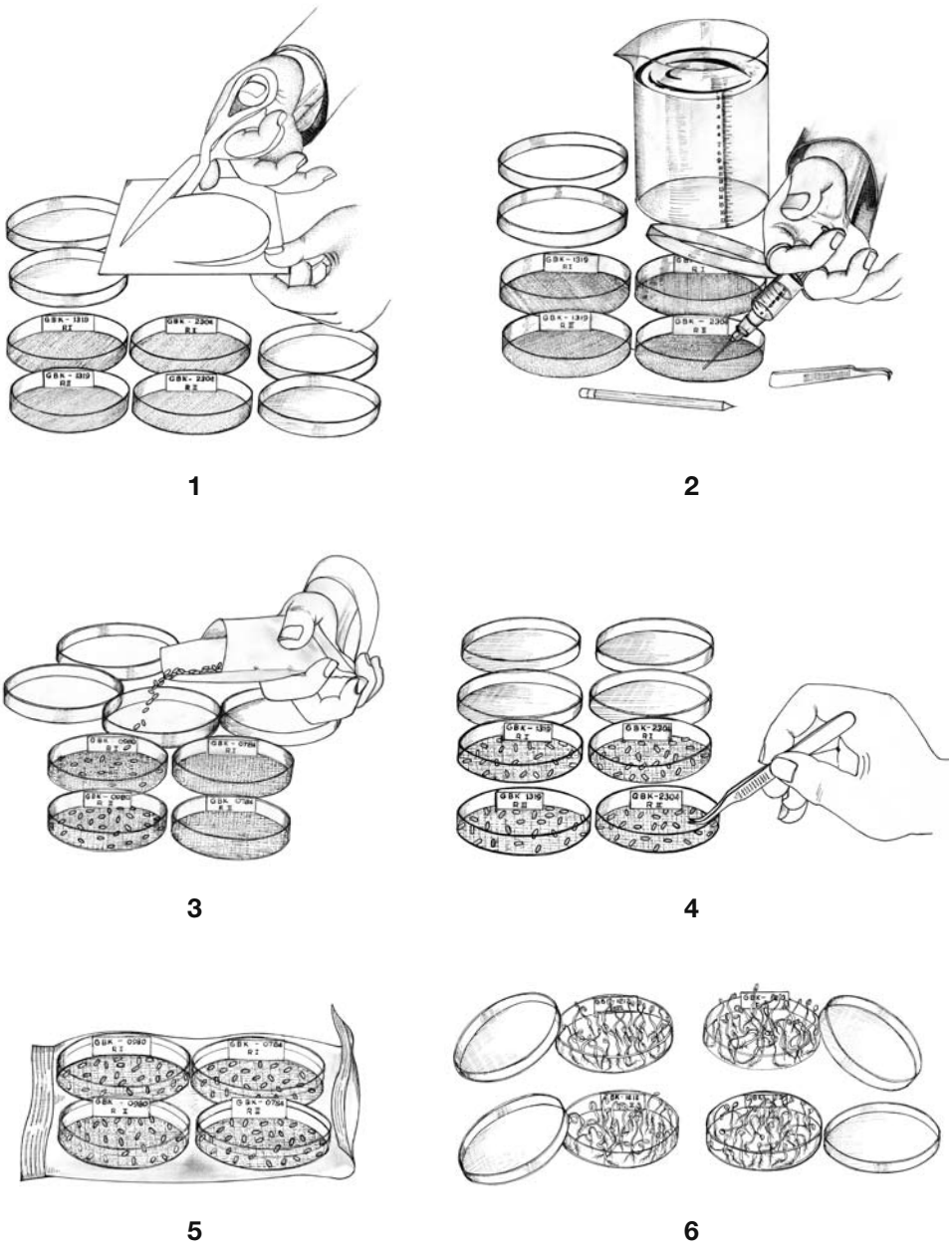


Figure 5.1. Seed germination testing on top of absorbent paper in Petri dishes.

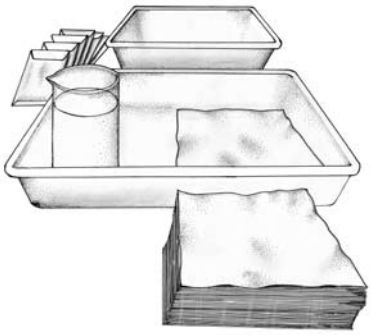
Between-paper method

This method is most appropriate for species with medium and large seeds between 2 mm and 1 cm in diameter, including many cereals, grain legumes and vegetables. Seeds are germinated between two layers of moist paper towel. Where possible, the towels should meet the specifications described above (e.g., non-toxic paper towelling from Seedburo Equipment Co., regular and heavy weight germination paper from Hoffman Manufacturing, Inc. and Grade 3663 seed testing paper from Whatman Plc.).

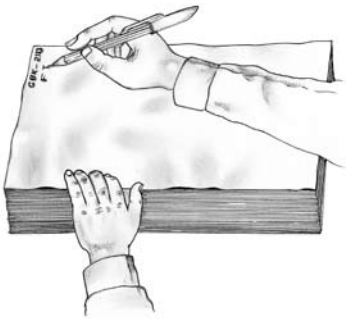
1. Cut the paper to a convenient size to hold one replicate of the seeds.
2. Label the paper at one end with the accession number, replicate number and the testing date. Use a pencil or permanent marker for labelling.
3. Moisten the paper with water.
4. Arrange the seeds in rows at regular intervals—about 4 cm from the top edge, leaving a 3–4 cm gap on the sides. Ideally, the distance between seeds should be at least three to five times the seed diameter.
5. Cover the seeds with another sheet of moist paper towel.
6. Roll the paper loosely from opposite the label end.
7. Use a paper clip or rubber band to hold the rolled papers and prevent them from falling apart.
8. Keep the rolls upright in a deep-bottom plastic tray.
9. Add a sufficient quantity of water to the tray (covering the bottom 3 cm of rolls).
10. Place the tray in an incubator or germinator maintained at the recommended temperature and run the test for the recommended period (see Table 5.1).
11. Keep the towels moist by spraying with water (use spray bottles) if necessary, especially when temperatures are high (25°–30°C).
12. Count the germinated seeds by unrolling the paper carefully to avoid tearing it or damaging the roots of young seedlings.
13. If some seeds have not germinated and appear to be dormant, treat with an appropriate technique to stimulate germination (see Table 5.1). Continue the test until all seeds have germinated or until no further germination has occurred after two consecutive counts.
14. Make a note of the seeds that did not germinate but are firm and sound at the end of first count, and those that failed to germinate and are presumed dead at the end of the germination test.

Figure 5.2 shows the stages in preparation of germination tests using paper towels.

The between-paper method is cheap and easy to prepare, but the seeds cannot be observed without unrolling the paper. Do not dry and reuse the paper for another test as it could carry fungal contamination from one test to another.



1



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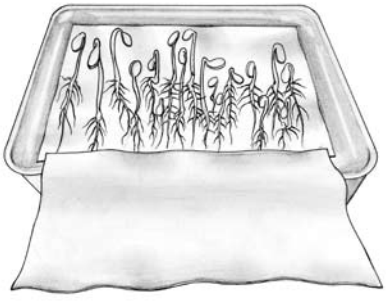

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Figure 5.2. Seed germination testing by between-paper method.

Germination in sand

This method is most appropriate for large seeds (with a diameter greater than 1 cm), which are difficult to germinate in Petri dishes or too heavy for the between-paper method.

1. Pack sterile, moist sand into pots or deep-bottom plastic trays with drainage. A single sheet of paper can be placed in the base of the tray to keep the sand from pouring out through the drainage holes.
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. Ideally, the distance between holes should be at least three to five times the seed diameter.
4. Prepare a plastic or wooden label with the accession number, date of sowing and replicate number, and place it in each tray.
5. Place one seed in each hole and cover the holes with sand.
6. Water the sand again by sprinkling to ensure that the sand layer is not displaced or the seeds are not disturbed when watering. Bottom-watering is better than top-watering—it is achieved by placing the test containers in larger plastic trays with water for about one hour.
7. Place the trays in appropriate light and temperature for the species.
8. Keep the substrate moist during tests by adding water, but do not over-water.
9. Run the test for the period recommended for the species and count the number of seeds that have sprouted.

Use fine sand for germination-testing. Quarry or river sand is better than shore sand. If shore sand must be used, wash it thoroughly to remove all salts. Clean the sand by pasteurising it (at 180°F or 82°C and 5 psi in three cycles of one hour each) before use.

Figure 5.3 shows the stages in testing germination using sand.

Agar method

Agar is an alternative substrate to paper, particularly for testing germination in small and medium-sized seeds. Agar dissolves slowly in hot water and forms a viscous solution, which forms a stiff jelly upon cooling.

1. Sterilize the surface of the containers by wiping them with 70–95% alcohol or soaking in 20% bleach or hot water at 55°C for 10–15 minutes.
2. Label 9-cm Petri dishes and their covers (for small seeds), or any other heat-resistant germination test containers, with accession number, number of replicate and testing date.
3. Prepare 1% agar solution (WA) by dissolving 1 g of agar powder in 100 ml of warm distilled water heated on a hot plate.
4. Allow the solution to boil until the agar is completely dissolved, then cool slightly to 50°C and pour into the labelled Petri dishes or the other containers. The thickness of the substrate should be twice the thickness of the seeds.

Agar stays moist for up to one month and is particularly suited as a substrate in dormancy studies. It is susceptible to contamination from airborne fungi and bacteria, however, and sterile conditions are required in the laboratory when using this substrate.

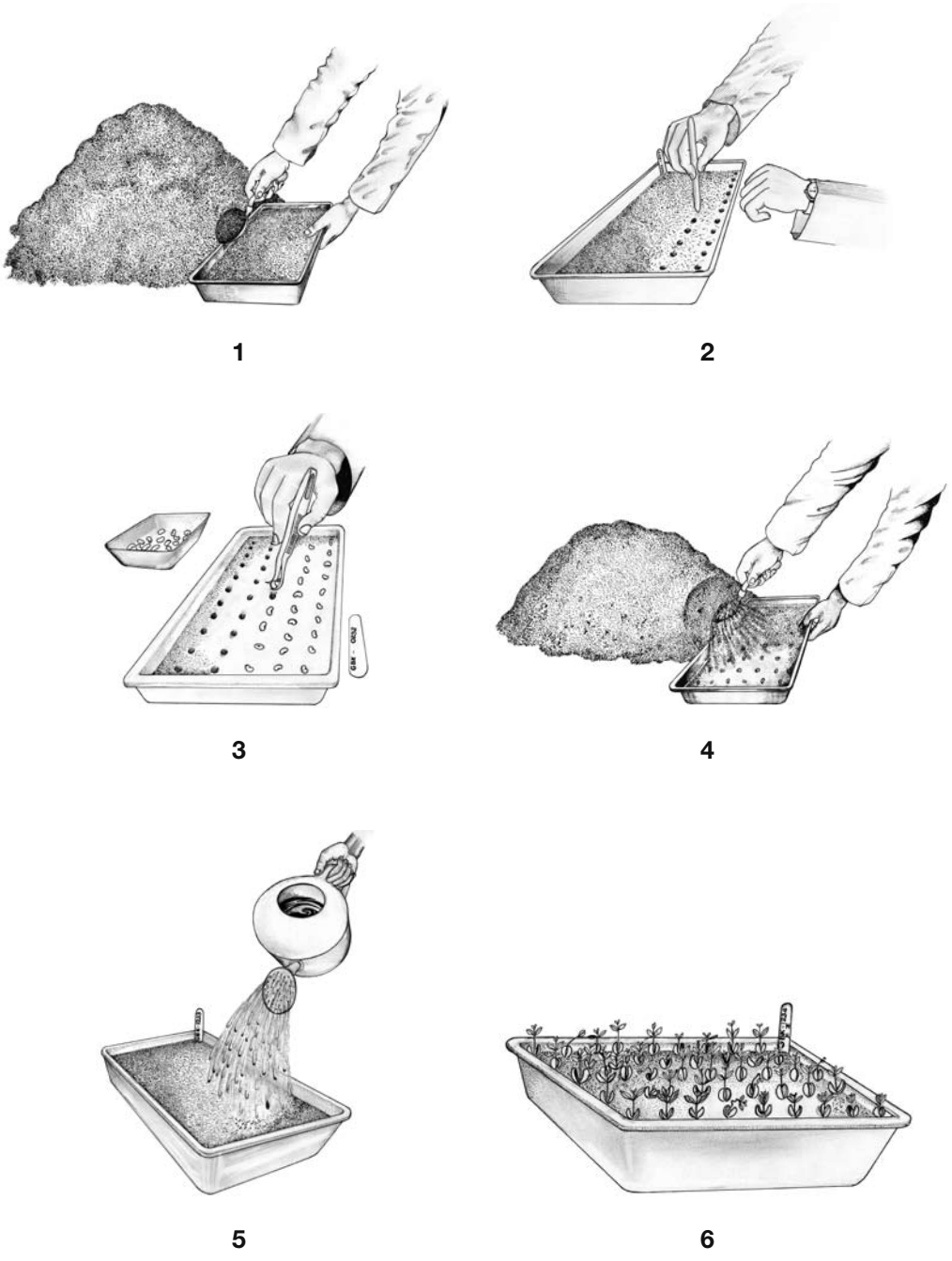

Figure 5.3. Seed germination testing in sand.

5. Arrange the seeds equidistantly on the surface of the agar.
6. Cover the dishes with their lids and place them in an incubator maintained at the recommended temperature for the species (see Table 5.1).
7. Run the test for the recommended period (see Table 5.1) and count the number of seeds that have germinated.

Step 3: Evaluation of germination tests

1. Seedlings removed during the course of germination testing are classified either as normal seedlings or abnormal seedlings (see Box 5.2).
   - Normal seedlings possess adequate root and shoot structures, which are essential for further development into plants.
   - Abnormal seedlings are incapable of further development and suffer deficiency, decay or weakness in their root or shoot systems.
2. It is important that germination tests are observed regularly and that normal seedlings and abnormal germinants are removed in order to allow other seedlings to develop in a less-crowded environment; it is also important to remove fungi-infected seeds in order to prevent the spread of infection. It is desirable to conduct an initial germination count after three or seven days, followed by a final count after seven or 14 days, depending on the species. Some species such as grasses require a test period of up to 21 or 28 days, and it is desirable to conduct an interim count at 14 days. Detailed germination procedures and periods for counting seedlings are provided in the ISTA and AOSA rules for seed-testing (see ISTA, 2005 and AOSA, 2005).
3. Record observations on the data sheet provided in Table 5.2.
4. Also record any abnormal seedlings or dead seeds removed during the first or interim counts (see Table 5.2)—they provide an indication of the progress of seed deterioration if a review is required at a later date.

Only those seedlings that are normal (those that demonstrate a capacity for sustained development under suitable conditions) are considered to have germinated. Abnormal seedlings should not be considered to have germinated.

Box 5.2. Seedling defects classified as abnormal (for more details, refer to ISTA [2003, 2005] or AOSA [2005]).

Roots

- Primary root stunted, stubby, missing, broken, split from the tip, spindly, trapped in the seed coat, with negative geotropism, glassy, decayed due to primary infection or with less than two secondary roots in monocots

Shoot (hypocotyl, epicotyl and mesocotyl)

- Short and thick, split right through, missing, constricted, twisted, glassy or decayed due to primary infection

Terminal bud/leaves

- Deformed, damaged, missing or decayed as a result of primary infection

Cotyledons

- Swollen, deformed, necrotic, glassy, separated or missing, and decayed due to primary infection

Examples of normal and abnormal seedlings in pea, groundnut, wheat and onion are shown as Figures 5.4 to 5.7.

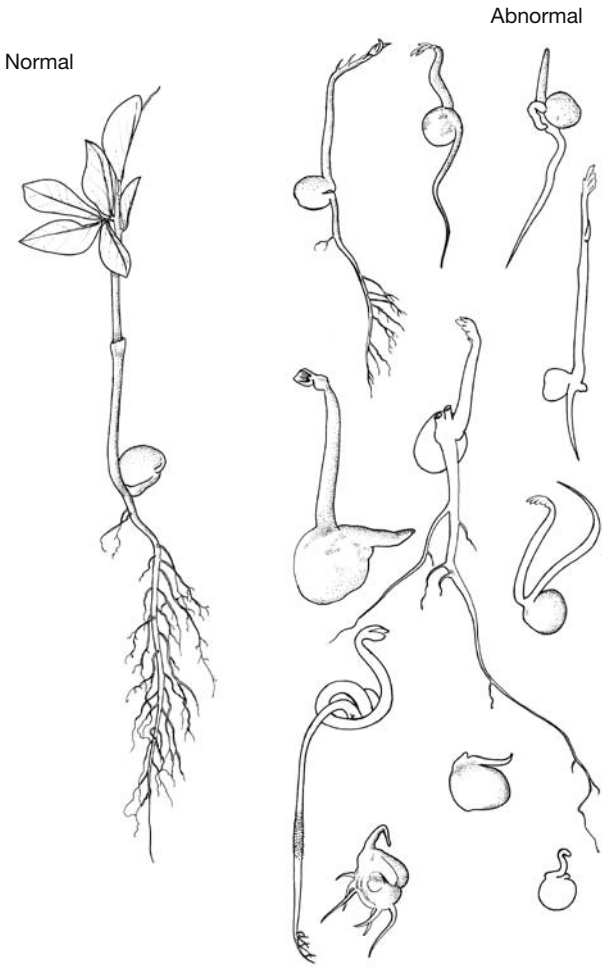

Figure 5.4. Seedling abnormalities in pea.

5. Upon completion of the germination test, count and record all un-germinated and dead seeds in each replicate.
6. Calculate the mean percentage germination of the accession from the results of all the replicates to determine the number of *normal seedlings* produced.
7. Repeat the germination test if the difference between the two replicates exceeds 10% or the maximum tolerance exceeds 2.5% probability (see Ellis et al., 1985).
8. Once a seed has been germinated, the resulting seedling can be discarded or transplanted for regeneration when the number of seeds in storage is critically low.

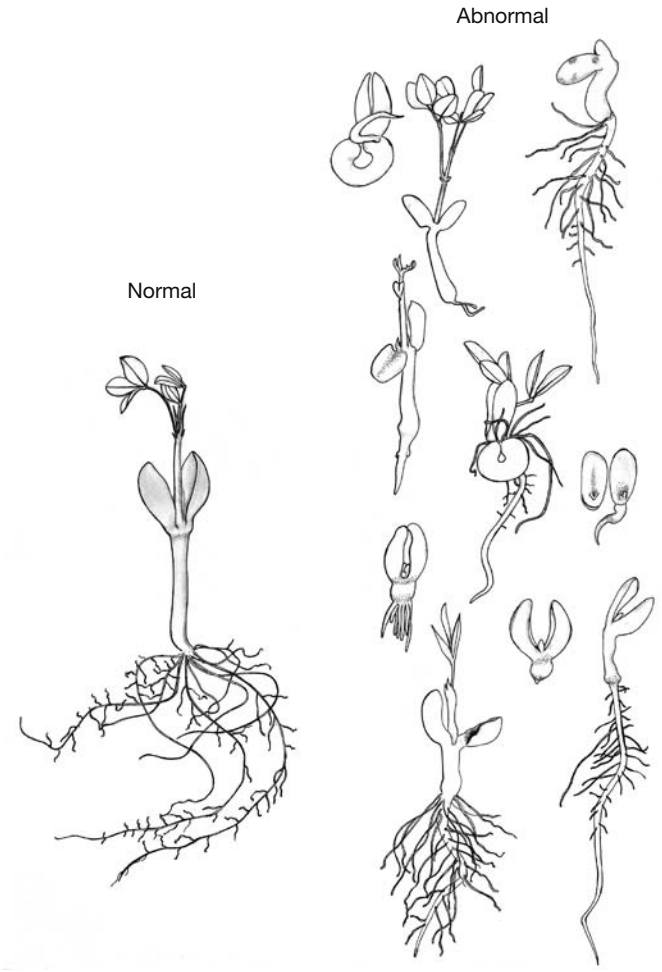

Figure 5.5. Seedling abnormalities in groundnut.

Why do some seeds fail to germinate?

Seeds fail to germinate either because they are dead or dormant. Dead seeds usually soften and rot during testing. To determine if seeds are dead or dormant, inspect the un-germinated seeds with a pair of tweezers to establish whether they are soft or firm. Un-germinated seeds found to have firm embryos are potentially viable. A high percentage of these seeds indicates that germination conditions were not optimal or that seeds are dormant.

Dormancy

Dormancy refers to the state in which viable seeds fail to germinate even under conditions normally favourable for germination.

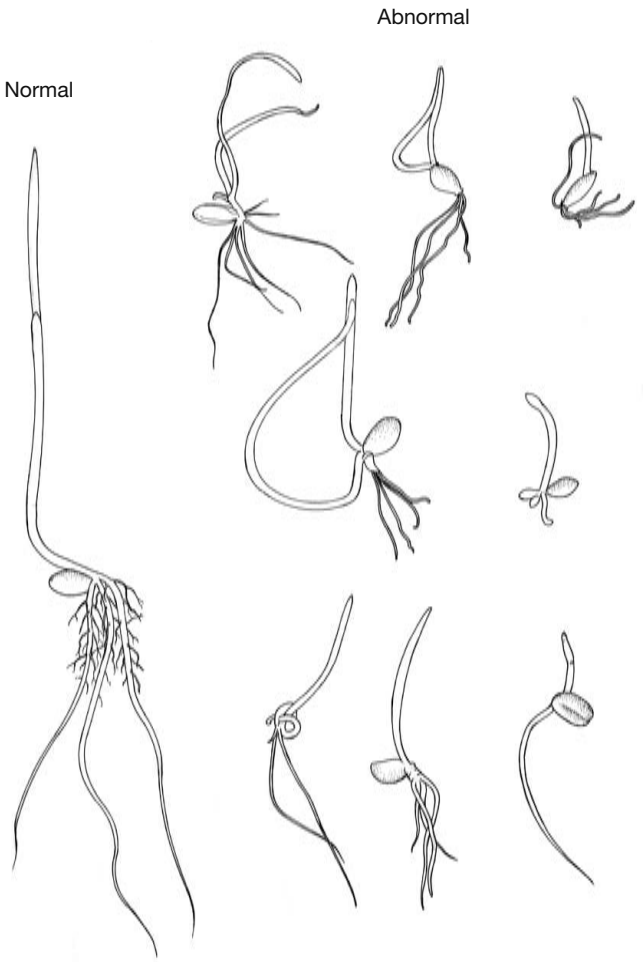

Figure 5.6. Seedling abnormalities in wheat.

How to determine if seeds are dormant

Seeds that remain hard, or absorb water but remain firm and in good condition, during germination tests are probably dormant. Seed dormancy is common in freshly harvested seeds and in many wild species of crop plants.

Types of dormancy

Seed-coat dormancy

Physical, chemical or mechanical conditions prevent uptake of moisture. Examples of seed-coat dormancy can be found in the

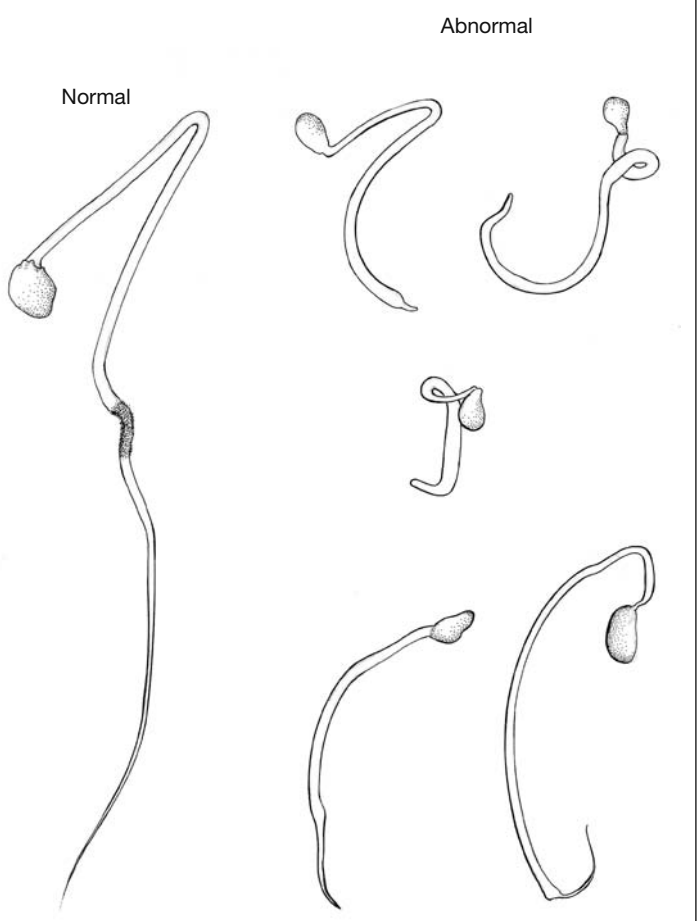

Figure 5.7. Seedling abnormalities in onion.

Anacardiaceae, Burseraceae, Cistaceae, Fabaceae, Geraniaceae, Malvaceae, and Rhamnaceae families.

Embryo dormancy

Inhibiting substances usually within the embryo or surrounding tissues prevent germination. Examples of embryo dormancy can be found in the Apiaceae, Iridaceae, Liliaceae, Papaveraceae and Ranunculaceae families.

In certain species, seed embryos are underdeveloped or not fully formed at seed dispersal. In these species, the embryo continues to

Table 5.2. Model data sheet to record germination results.

Crop/species: Substrate:
Accession number: Temperature:
Batch reference number: Light:
Date of storage: Special treatments:
Date of testing: Incubation time:

Replication		Normal seedlings				Total	Remarks
		I	II	III	IV		
No. of seeds tested						**Total**	**Remarks**
Date	**Days**						
Total germinated							
Abnormal							
Hard/dormant							
Dead							
Germination (%)							

grow after dispersal, and germination is prevented until the embryo reaches a species-specific critical length. Examples can be found in the Annonaceae, Apiaceae, Orchidaceae, Orobachaceae and Ranunculaceae families.

Dormancy can be also caused by a combination of impermeable seed or fruit coats and physiologically dormant embryos. For germination to occur, both types of dormancy must be broken. The order in which each type of dormancy must be broken depends on the species. Examples are *Ceanothus* (Rhamnaceae), *Tilia* (Tiliaceae) and *Rhus* (Anacardiaceae).

How to determine the type of dormancy

If removal of the seed coat does not result in germination, the dormancy mechanism is located in the embryo itself.

Dormancy-breaking treatments

In some seeds that are dormant at harvest, dormancy breaks down naturally over time. Other species require some form of pre-treatment. There are several methods used for specific genera.

Breaking seed-coat dormancy

Puncturing or scarifying the seed coat by piercing, nicking, chipping or filing with a knife, needle or sandpaper are preferred procedures to overcome seed-coat dormancy.

- Manual scarification is effective at any point on the seed coat, but the micropylar region should be avoided as it is the most sensitive part of the seed where the radicle is located (see Figure 5.8).

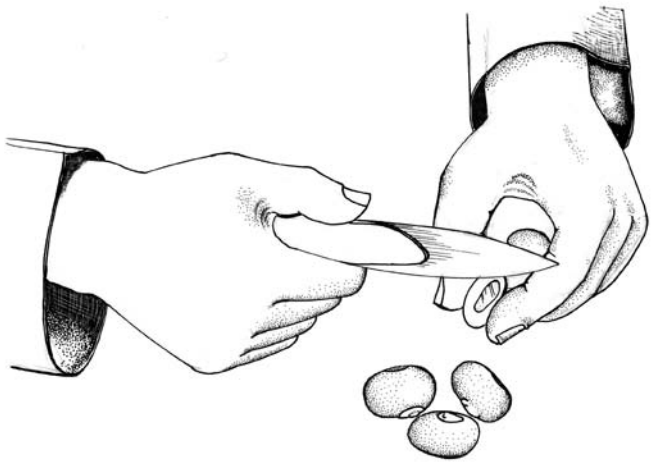

Figure 5.8. Manual scarfication of seed coat.

- If seed-covering structures prevent growth of the embryo, remove them to allow germination.
- If the seed coat contains inhibitors that prevent or delay germination, they can be leached out by placing the seed under running water for several hours or soaking the seed in a large volume of water that is changed every six to 12 hours.
- ISTA also recommends using concentrated sulphuric acid for 2–45 minutes depending on the species to scarify the seed coat. This method is expensive and dangerous, however, and should be followed with caution.
- To remove the waxy covering and allow imbibition, place the seeds in water at 75°C for three to six minutes. Care must be taken not to use high temperatures for long periods or boil the seeds.

Breaking embryo dormancy

There are several recommended treatments to overcome embryo dormancy (see Table 5.1). These include pre-chilling (also called cold stratification) for temperate and high-altitude species from the tropics;

preheating; application of gibberellic acid (GA_3) at low concentrations; addition of potassium nitrate (KNO_3) to the substrate; and light.

Pre-chilling (cold stratification)
Seeds are placed in containers on a moistened germination substrate and kept at 3° to 5°C in a refrigerator for seven days. For more dormant seeds, the treatment may be extended to 14 days. Once the stratification is complete, the containers are removed to incubators and seeds are allowed to germinate in recommended conditions.

Preheating
Seeds are treated at a temperature not exceeding 40°C for up to seven days with free air circulation before germination in recommended conditions.

Gibberellic acid
Germination test paper is moistened with a 0.05% solution of gibberellic acid (GA_3), prepared by dissolving 500 mg of GA_3 in 1 l water. Germination is then continued in recommended conditions.

Potassium nitrate
A 0.2% solution of potassium nitrate (KNO_3)—prepared by dissolving 2 g KNO_3 in 1 l water—is used to moisten the germination paper at the beginning of the test. Germination is continued in recommended conditions.

Light
Light may or may not be required for germination, depending on the species. When using constant temperatures for germination of species where light is required, the tests should be illuminated for at least eight hours of every 24-hour cycle. When alternating temperatures are used, any necessary application of light should coincide with the high-temperature cycle. Light intensity should be 750–1250 lux from cool, white lamps.

Many of the methods described above are specific to genera. Recommended dormancy-breaking treatments for common crops are given in Table 5.1. For information on other species, refer to Ellis et al. (1985).

Algorithm for developing suitable germination test procedures for species for which no information is available

Step 1

- Determine if seed coats are impermeable by checking imbibition of seeds placed on moist filter paper overnight. If seeds have

not imbibed water, scarify the seed coats using a scalpel blade and observe again after 12 hours. Proceed to germination when seeds have imbibed the water.

Step 2

- If the first step does not result in full germination and if the accessions are of temperate origin, test at constant temperatures of 15°C and 20°C. For accessions of tropical origin, use constant temperatures of 20°C and 25°C.
- If the accession origin is unknown or doubtful, test at 15°, 20° and 25°C.
- In all cases, apply light for 12 hours per day.

Step 3

- If the second step has not resulted in full germination, test a further sample of seeds in alternating temperatures 25°/10°C (12 hours and 12 hours) for accessions of temperate origin and 35°/20°C (12hours and 12hours) for accessions of tropical origin.
- If light is applied for 12 hours per day, it should coincide with the upper temperature cycle.
- If the accession's origin is unknown or doubtful, test a sample of seeds at each temperature.

Step 4

- If the third step has not resulted in full germination, add 0.1–0.2% potassium nitrate (KNO_3) to the test substrate in the most successful temperature regime determined in steps 2 and 3.

Step 5

- If the fourth step has not resulted in full germination, pre-chill the seeds at 2°C to 6°C for eight weeks and test for germination in the most successful regime determined in steps two through four.

Step 6

- If full germination is not obtained, estimate viability using the tetrazolium test described below. The results of this test will indicate if the failure to achieve full germination is due to the presence of dead seeds.
- If the tetrazolium test indicates that dormancy is not broken and seeds are viable, try other dormancy-breaking treatments such as gibberellic acid (GA_3) or pre-heating at 40°C for three to seven days.

Tetrazolium test for seed viability

The tetrazolium test can be used as a backup procedure to identify viable but dormant seeds that have failed to germinate at the end of a germination test. The procedure for this test is indicated below.

The tetrazolium test is not an absolute test of seed viability. To gain reliability, the test must be compared with the results of germination tests for each species.

Preconditioning

1. Remove the seed-covering structures (glumes, etc.).
2. Precondition the seeds by soaking in water or by placing them in a moist medium at 30°C. No preconditioning is necessary when un-germinated seeds are evaluated at the end of a germination test.

Preparing tetrazolium chloride solution

The tetrazolium solution should be between pH 6 and 8 to achieve best results. To prepare 1 litre of buffered 1% tetrazolium chloride solution:

1. Dissolve 3.631 g of potassium dihydrogen phosphate (KH_2PO_4) in 400 ml of distilled water.
2. Dissolve 7.126 g of disodium hydrogen phosphate ($Na_2HPO_4.2H_2O$) in 600 ml of distilled water.
3. Mix the two solutions to prepare the buffer.
4. Dissolve 10 g of 2,3,5,-triphenyl tetrazolium chloride in the 1 litre of buffer solution.

To produce 0.5% tetrazolium solution, mix one part stock solution with one part distilled water. Tetrazolium chloride solution should be stored in dark and cold conditions for short periods.

Staining

1. Bisect the seeds longitudinally through the embryo with a razor blade.
2. Discard half of each seed and place the other half in the staining solution at the recommended concentration (see Table 5.3) in a glass vial.
3. Place the vials in an incubator in a dark area at the recommended temperature and duration for each species (see Table 5.3).
4. After staining, wash the seeds several times in distilled water to remove excess stain.
5. Immerse the seeds in lactophenol solution (1 litre of lactophenol prepared from 200 ml phenol, 200 ml lactic acid, 400 ml glycerine, and 200 ml water) for one to two hours before evaluating the seeds.
6. Evaluate the seeds for a staining pattern under a low-powered binocular microscope; viable tissues stain bright red. Pink and very dark red stains indicate dead tissue.
7. Classify the seeds into three categories depending on staining pattern:
   - completely stained seeds that are viable;
   - completely unstained seeds that are nonviable; and
   - partially stained seeds that will produce either normal or

Table 5.3. Concentration, temperatures and period of staining with tetrazolium solution (for Annex I crops of the International Treaty on PGRFA).

Crop	Species	Preconditioning	Staining
Barley	*Hordeum vulgare*	Imbibe or soak, 6–18h	0.5%, 3h, 30°C
Beans	*Phaseolus* spp.	Imbibe 18–24h, then soak, 2–3h	0.5–1%, 6–24h, 30°C
Brassica	*Brassica* spp.	Imbibe or soak, 16–18h	0.5–1%, 3–6h, 30°C
Chickpea	*Cicer arietinum*	Imbibe or soak, 18h	1%, 6–24h, 30°C
Cowpea	*Vigna unguiculata*	Soak, 22h	0.5–1%, 16–24h, 30°C
Eggplant	*Solanum melongena*	Imbibe or soak, 18h	0.5–1%, 6–24h, 30°C
Faba bean	*Vicia faba*	Soak, 22h	0.5–1%, 16–24h, 30°C
Finger millet	*Eleusine corocana*	Soak, 18h, 5°C	0.5%, 3h, 30°C
Lentil	*Lens culinaris*	Imbibe, 18h, then soak, 2–3h	1%, 6–24h, 30°C
Maize	*Zea mays*	Imbibe or soak, 18h	0.5–1%, 2–6h, 30°C
Pea	*Pisum sativum*	Imbibe 18–24h, then soak, 2–3h	0.5–1%, 6–24h, 30°C
Pearl millet	*Pennisetum glaucum*	Imbibe or soak, 6–18h	0.5–1%, 6–24h, 30°C
Rice	*Oryza sativa*	Imbibe or soak, 18h	0.5%, 3h, 30°C
Rye	*Secale cereale*	Imbibe or soak, 6–18h	0.5%, 2–3h, 30°C
Sorghum	*Sorghum bicolor*	Imbibe, 16h, 30°C	0.5–1%, 0.5–1h, 40°C
Sugar beet	*Beta vulgaris*	Imbibe or soak, 16–18h	1%, 24–48h, 30°C
Sunflower	*Helianthus annuus*	Imbibe or soak, 18h	0.5–1%, 3–6, 30°C
Triticale	*Triticosecale*	Imbibe or soak, 6–18h	0.5%, 2–4h, 30°C
Wheat	*Triticum aestivum*	Imbibe or soak, 6–18h	0.5%, 2–4h, 30°C

abnormal seedlings, depending on the intensity and pattern of staining (see ISTA 2005 for more information).

Figures 5.9 and 5.10 show patterns of tetrazolium staining in dicot and monocot seeds, respectively.

Documentation

Documenting viability data is crucial for the efficient management of germplasm collections as it enables genebank staff to make informed decisions regarding the timely regeneration of material (see Chapter 8). Suggested descriptors to document accession-level information on viability (germination) testing include the following:

- Number of seeds tested per replicate
- Number of replicates
- Method of germination testing
- Date of germination testing
- Duration of testing (or days of first and final counts)
- Number of germinated seeds at first count

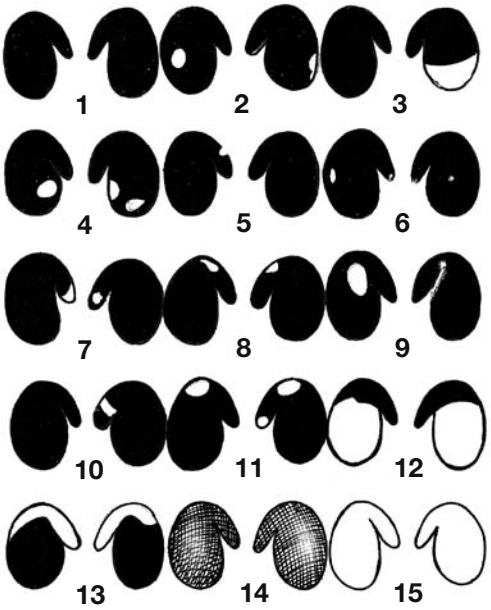

Figure 5.9. Staining pattern after tetrazolium test in dicot seeds. Illustrations depict both sides of seeds. Numbers 1–6 are germinable seeds and numbers 7–15 are nongerminable seeds (adapted from AOSA, 2005).

- Dormant/hard seeds at first count (%)
- Special treatments for dormancy breaking (if any)
- Final germination (% normal seedlings)
- Abnormal germination (%)
- Dead seeds (%)
- Tolerance levels for statistical accuracy

Further reading

Association of Official Seed Analysts. 2005. Rules for testing seeds. Association of Official Seed Analysts, USA.

Baskin, C. C. and Baskin, J. M. (1998) Seeds: Ecology, Biogeography and Evolution of Dormancy and Germination. Academic Press, San Diego, USA.

Ellis, R.H., Hong, T.D and Roberts, E.H. 1985. Handbook of seed technology for genebanks. Volume 1: Principles and methodology. Handbooks for Genebanks. No. 2. IBPGR, Rome, Italy.

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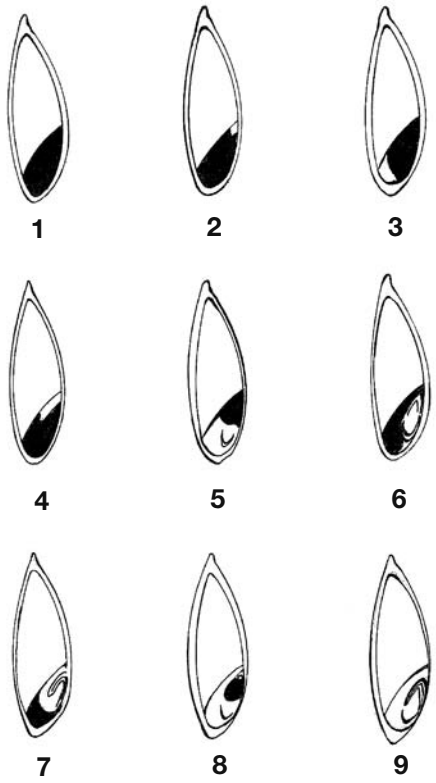

Figure 5.10. Staining pattern after tetrazolium test in monocot seeds. Numbers 1–4 are germinable seeds and numbers 5–9 are nongerminable seeds (adapted from AOSA, 2005).

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

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5.2 Seed health testing

What is seed health?

Seed health refers to the disease status of a seed sample and the presence or absence of disease-causing organisms and pests.

What is seed health testing?

Seed health tests determine the status of a seed sample, seed lot or accession with regard to diseases affecting that crop or wild species.

Why is seed health testing important?

Crops are frequently infected with a range of common seed-borne pathogens that may not be visible or easily recognized during seed collection. Seed-borne inoculums reduce storage longevity and cause poor germination or field establishment. Seed-borne inoculums also promote disease in the field, reducing the value of crops. Exchange of infected seeds may allow spread of diseases and pests into new regions. Genebanks should ensure that seeds prepared for conservation are free from seed-borne diseases and pests.

Common seed-borne pests and pathogens

There are four main types of common organisms that are carried in seeds and affect a wide range of crops:

- Fungi
- Bacteria
- Viruses
- Insects

Specific methods for detecting pathogens vary by organism and host, and specific methods are required for accurate identification of most pathogens.

Methods of detecting pests and pathogens

Seed-health standard

Examine a representative sample of seeds for the presence of pathogens using one or more of the following methods. Usually, a sample of 400 seeds in replication of 100 seeds each is drawn for examination. Sample size can be decreased for small seed lots.
If the percentage of seeds infected is greater than 5%, the seed lot can be considered unsuitable for conservation.

Visual examination

The simplest method to detect diseases and pests is to examine dry seeds with the naked eye or under a low-powered microscope. This method reveals freely moving insects, eggs, mites, fungal fructifications like sclerotia, galls, smut balls, bacterial masses and infected plant debris. Examination of dry seeds under ultraviolet or near-ultraviolet light reveals infections of certain fungi and bacteria through emission of fluorescence.

Seedling evaluation

Seeds should be planted in sterilized soil in a screenhouse. Seedlings should be observed immediately after germination and any plants exhibiting virus-like symptoms such as leaf mottling, curling or yellowing should be sampled and tested for viruses (see

below). Seedlings infected with bacteria or fungi may die and should be examined further in a laboratory, and the samples should be plated for identification of the pathogen (see below).

If infection is suspected but no symptoms have been observed after the second true leaf has emerged, it may be necessary to carry out serological tests for latent or symptom-less infection by viruses. Most legume viruses will express conspicuous symptoms at the seedling stage.

Seed-washing technique

This is useful for testing surface-borne, contaminating fungi such as smuts, bunts, downy mildews, powdery mildews and rusts.

1. Place 2 g of the seed sample in a test tube, add 2 ml of sterile water and mix well for five to ten minutes.
2. Centrifuge the supernatant solution at 200 rpm for ten minutes and observe the sediments under a microscope for fungal structures.

Incubation methods

The blotter and agar-plate methods are simple and inexpensive ways to detect seed-borne fungi that respond to sporulation.

Blotter test

Blotter tests are similar to germination tests in that seeds are placed on moistened layers of absorbent paper and incubated under conditions that promote fungal growth.

1. Line the base of sterilized Petri dishes with three layers of absorbent paper moistened with sterile water.
2. Drain off excess water and place 20–25 seeds manually with forceps.
3. Evenly space the seeds to avoid contact.
4. Incubate the seeds under near-ultraviolet light in alternating cycles of 12 hours light/darkness for seven days at 20°±2°C.
5. Examine the Petri dishes under a stereo-binocular microscope for fungi developing on the seeds.

Profuse seedling growth may make interpretation difficult. This may be overcome by adding 2,4-D sodium salt to provide a 0.2% moistening solution.

Agar-plate method

This is the most common method used for identifying seed-borne fungi. Different fungi and even different strains of the same fungi require different media for growth and sporulation. Near-ultraviolet light with a wavelength 300–380 nm (also called black light) may be required. Simple media include a combination of vegetables, carbohydrate or sugar sources and agar, and can be made by

combining boiled and mashed vegetables with agar when commercial mixes are unavailable. The most commonly used media are potato dextrose/sucrose agar and oatmeal agar.

1. Prepare the medium by mixing 1 g potato dextrose agar powder in 100 ml distilled water.
2. Sterilize the mixture in an autoclave for 15–20 minutes and cool to 50°C.
3. Carefully pour the mixture into sterile Petri dishes, lifting the lid enough only to pour in the agar to avoid contamination.
4. Allow it to cool and solidify for 20 minutes.
5. Surface-disinfect the seeds by pre-treating them for one minute in a 1% sodium hypochlorite (NaOCl) solution prepared by diluting 20 parts domestic bleach (5% NaOCl) with 80 parts water.
6. Place approximately ten seeds (depending on size) on the agar surface with forceps.
7. Incubate the Petri dishes at 20°–25°C for five to eight days.
8. Identify the seed-borne pathogens on the basis of colony and spore characteristics.

Sometimes bacterial colonies develop on the agar and inhibit fungal growth, making identification difficult. This can be overcome by adding an antibiotic such as streptomycin (500 ppm) to the autoclaved agar medium after it cools to 50°–55°C.

Polymerase chain reaction (PCR) method

PCR is an *in vitro* technique to amplify a small quantity of a specific nucleotide sequence exponentially in the presence of a template sequence with two oligonucleotide primers that hybridize to opposite strands and flank the region of interest in the target DNA. The reaction is cycled, involving template denaturation, primer annealing, and extension of the annealed primers by DNA polymerase until enough copies are made for further analysis. PCR can allow the detection of very small amounts of a pathogen in a sample by amplifying the pathogen sequences to a detectable level. PCR is especially useful to detect diseases because of its speed and accuracy, but it is an expensive technique—it can be used to detect any organism that has DNA by using positive and negative controls for comparison. Once the sequence of the organism is known, specific probes can be made to detect strains of pathogens.

Nucleic acid hybridization assays (called southern and northern blotting), in which DNA or RNA is transferred from an electrophoresis gel onto a membrane and then the nucleic acids are detected with a labelled probe, can also be used. The nucleic acid spot hybridization (NASH) technique, in which a labelled DNA pathogen hybridizes directly to the pathogen DNA immobilized on a nylon membrane, can also be used

without going through the PCR stage. These techniques are constantly being refined and new procedures are becoming available for specific pathogen detection. For more information, refer to Albrechtsen (2005).

Serological and other methods

Enzyme-linked immunosorbent assay (ELISA)

ELISA is a diagnostic method that uses proteins called antibodies to detect plant pathogens. This assay is based on the ability of an antibody to recognize and bind to a specific antigen—a substance associated with a plant pathogen. The antibodies used in diagnostics are highly purified proteins produced by injecting a warm-blooded animal (like a rabbit) with an antigen associated with a particular plant disease. The animal reacts to the antigen and produces antibodies, which recognize and react only with the proteins associated with the causal agent of that plant disease. Colour changes on the unit's surface indicate a positive reaction (disease present).

There are many different types of ELISAs that can detect the presence of protein. A detailed description of these is beyond the scope of this publication and genebank staff members are advised to refer to Albrechtsen (2005). However, the general procedures for two most common methods—antigen-coated plate (ACP-ELISA) and tissue-blot immunoassay (TBIA)—are given in Annex II. For more details, refer to Lin et al. (1990).

Indicator plant method

This is especially useful for detecting bacteria and viruses. Seed extracts are prepared and inoculated on indicator plants like tobacco. The pathogens are identified based on the symptoms that develop. Indicator plants can also be used to separate different viruses by virus-host specificity.

Documentation

Suggested descriptors to document accession-level information on seed health-testing include the following:

- Source of the material for testing
- Type of material (leaf, stem, root, seeds)
- Number of plants sampled and tested per replicate
- Number of replicates
- Organisms tested for
- Method of testing
- Date of test
- Duration of test, if appropriate
- Diseases identified
- Incidence of each disease (%)

Further reading

Albrechtsen, S.E. 2005. Testing methods for seed-transmitted viruses: Principles and protocols. Oxford University Press, Oxford, UK.

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

Lin, N.S., Hsu, Y.H. and Hsu, H.T. 1990. Immunological detection of plant viruses and mycoplasm-like organisms by direct-tissue blotting in nitrocellulose membranes. Phytopathology, 80: 824–828.

5.3 Seed testing for inadvertent introduction of transgenes

What are transgenes?

Transgenes are genes that are introduced into another organism or species through recombinant DNA techniques. Transgenic plants carry transgenes in their genomes and transmit them to their progeny through normal reproduction.

Why determine the presence of a gene/transgene?

One of the most important components of proper genebank management is testing for the presence of a gene or phenotype. This is critical for various phytosanitary requirements, but is also becoming important for the detection of transgenes. There are a number of reasons why it is important to detect the presence of a gene/transgene in a genebank accession. While not an exhaustive list, these include:

- regulatory issues, especially related to phytosanitary or biosafety, where the country of import, and potentially the country of export, requires reporting on the presence of such genes;
- situations in which the presence of such a gene/transgene could affect intellectual property rights either in the country where the genebank is located or in a country where the accession is to be sent; and
- social issues necessitating that genetic identity be stated or that certain genes/transgenes should be limited.

When should one detect the presence of a gene/transgene?

It is generally accepted as unwise for crops containing transgenes to be incorporated into germplasm collections. The risk of inadvertent inclusion of transgenes can be classified as follows:

- High probability: typically out-crossing crops with sexually compatible relatives on which extensive research in the field or commercial release is ongoing.
- Low probability: typically crops which are highly self-pollinating, multiplied vegetatively or crops for which genetic engineering has either not been done or is in its very early stages.

- Medium probability: the remainder of crops.
- Immediate attention: crops with transgenes that are already commercially distributed.
- Near-future attention: experimental field work is ongoing or expected within one to three years.
- Long-term attention: crops for which no significant work has been done in the field.

Genebanks should take proactive steps to limit the risk of exotic genes, including transgenes, in their *ex situ* collections. Accessions that do not require testing include:

- species where no transgenic events (commercial or research) have occurred;
- accessions for which there were no commercial transgenes present at the time of acquisition (such as maize prior to 1996) or no transgenics near the collecting site; and
- accessions for which there have been transgenic events, but good management practices have been followed in the accession process.

In 2004, the Genetic Resources Policy Committee (GRPC) and the Science Council of the CGIAR organized a technical workshop to explore the ways and means to handle unintentional presence of transgenes in germplasm collections, with the goal of providing technical inputs into a process that would enable CGIAR Centre genebanks to draw up procedures aimed at preventing the unintentional introgression of transgenes into the collection. Further to this workshop, a guiding principle was prepared and adopted by the GRPC. For more information on this subject consult the Policies and Ethics section of Bioversity's website (www.bioversityinternational.org/About_us/Policies_and_Ethics/index.asp; last visited 20 December 2006). These guiding principles were also considered at the Third Session of the Intergovernmental Technical Working group on Plant Genetic resources for Food and Agriculture held at FAO Rome October 26-28 October 2005. Further information about that meeting is available from the Commission web site http://www.fao.org/waicent/FaoInfo/Agricult/AGP/AGPS/pgr/ITWG3rd/docsp1.htm

Procedures to prevent unintentional gene flow from genetically modified organisms (GMOs)

Transgenes and conventional genes are subject to the biological processes of mutation, gene flow, introgression, recombination and natural selection. Therefore, best practices for preventing introgression of conventional genes also provide an appropriate basis for preventing introgression of transgenes.

Germplasm is most at risk from gene flow during regeneration (see Chapter 8) and controlling gene flow is essential to ensure genetic integrity. To reduce the risk in crops where transgenes are commonly part of new cultivars, it is recommended that regeneration be carried out in isolation from any areas where transgenic crops are likely to be grown.

Information on crops' transgenic status is essential to determine what measures, if any, are needed to confirm that germplasm is free of transgenes. It is recommended that:

- all results be made publicly available as soon as they have been confirmed;
- all procedures and supporting information be presented;
- the appropriate authority in the country of origin be informed in cases where transgenes are detected; and
- for commercially released genetically modified crops and crops in experimental development, genebanks maintain a database of crops and their status in transgenic research.

Once an accession has either been determined to not require testing or has tested negative, follow appropriate regeneration and maintenance procedures to maintain genetic integrity, as for all accessions.

Procedures for testing for presence of GMOs

The two basic methods to detect the presence of a gene/transgene are ELISA and PCR amplification. Both methods have already been described and are robust, although each has advantages and disadvantages. For example, ELISA detects the presence of a gene product (protein) and thus requires an expressing gene. Test kits are commercially available for most commercial events, which can be used in the field. On the other hand, PCR can detect non-expressing gene sequences, in almost all tissues, but it is more difficult to perform and therefore not practical in the field. In most cases, the detection of a positive result using one method should be confirmed with a second method. If the materials are being analyzed at the molecular level for fingerprinting or diversity studies, an additional test for the presence of a transgene can be performed at minimal cost.

The genes/transgenes that should be used in such tests include the current commercialized major events for the species. These can normally be found on the Internet and are indicated in the tests provided by commercial testing services (either as ELISA kits or PCR services). These will change as new transgenic events are introduced into the market or events become obsolete and are

removed, although the need to test may continue for some time. The number of seeds in any accession may limit the level of detection. More information and technical guidance on sampling and detection of GMOs can be found at www.europa.eu.int/comm/environment/biotechnology/pdf/recom2004_787.pdf.

An updated list of validated methods is also available at http://biotech.jrc.it.

Documentation

Suggested descriptors to document accession-level information on the presence of transgenes include the following:

- Source of the material for testing
- Type of material (leaf, seedling, seed)
- Number of plants sampled and tested per replicate
- Number of replicates
- Transgenes tested
- Method of testing
- Date of test
- Duration of test, if appropriate
- Transgenes identified
- Incidence of each transgene (%)

6. SEED PACKAGING AND STORAGE

6.1 Seed packaging

What is meant by seed packaging?

Seed packaging involves placing a counted or weighed sample of seeds into a container, which is then hermetically sealed for subsequent storage (see Flowchart 6.1).

Why are seeds packaged?

Seeds are packaged to:

- prevent absorption of water from the atmosphere after drying;
- keep accessions separate and avoid mixing them; and
- prevent contamination from insects and diseases.

When should seeds be packaged?

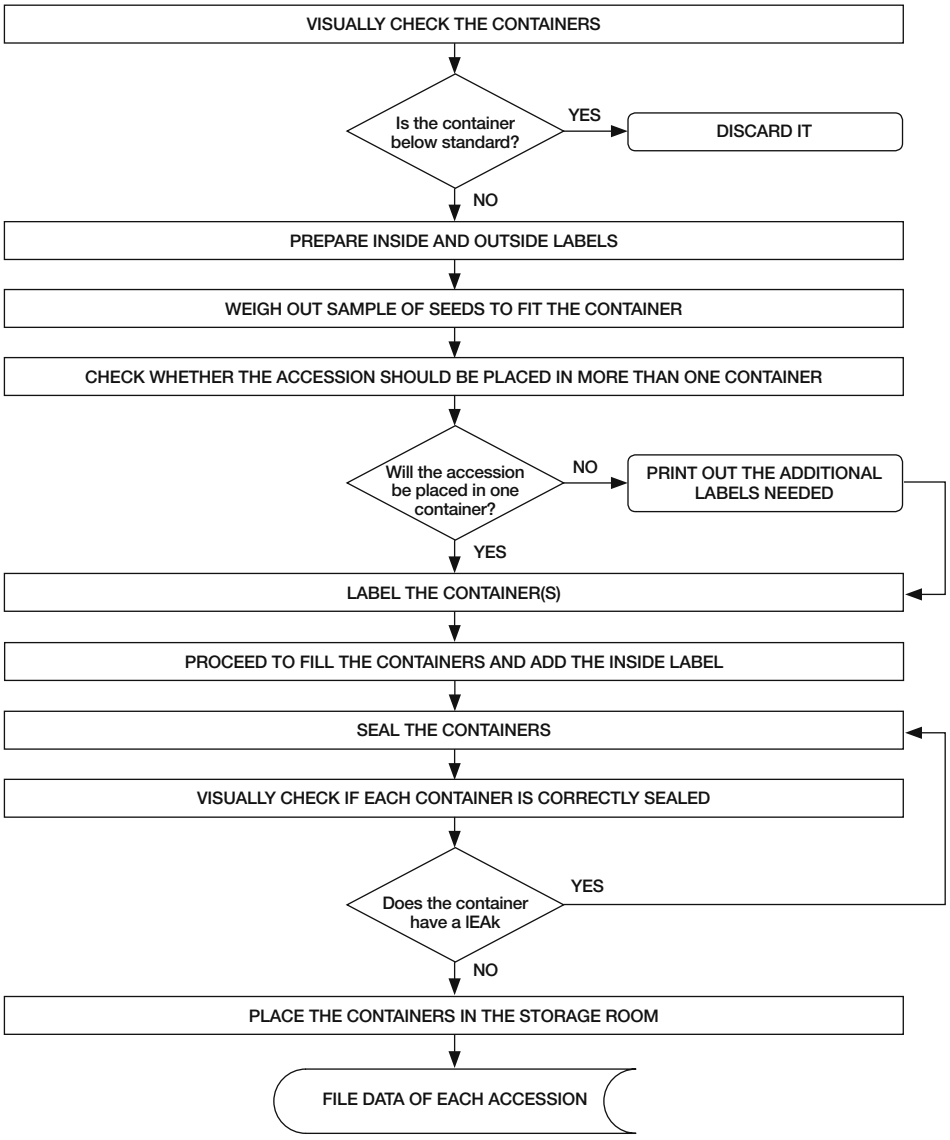
The best time to package seeds is immediately after moisture content has been determined to be within the required limits for safe storage. Dry seeds will reabsorb moisture from more humid ambient air. Therefore, seeds should be packaged in waterproof containers and hermetically sealed without delay following removal from the drying room or cabinet.

Types of container

Different types of container are available for packaging; the choice depends on storage conditions and species. It is important that the packing material be completely impermeable to water and suitable for long-term use. Frequently used containers include glass bottles, aluminium cans, laminated aluminium foil packets and plastic bottles.

Different types of container each have advantages and disadvantages. Glass bottles are good but can easily break. Aluminium cans are difficult to reseal once they have been opened. Aluminium foil packets can be resealed and occupy less space than other containers, but seeds with

Flowchart 6.1. Seed packaging.



sharp projections can pierce them and moisture can leak inside. Plastic bottles and aluminium cans with lids are moisture resistant but not moisture proof unless they have a tight rubber seal. They should be used with caution if the RH of the storage room is not controlled.

Laminated aluminium foil packets are the most commonly used containers in genebanks as they occupy little space and it is easy to reseal them. Aluminium foil packets to be used in genebanks should have the following specifications:

- an outer layer of 17 g m^{-2} Melinex, 4 g m^{-2} lacquer;
- a middle layer 33 g m^{-2} (12 µm) aluminium foil, 4 g m^{-2} lacquer; and
- an inner layer of 63 g m^{-2} polyethylene.

Testing quality of containers

The quality and sealing capacity of containers can be tested as follows:

1. Fill the containers with regenerated self-indicating silica gel and seal it in the same way in which seeds are stored.
2. Accurately determine the weight of the containers with an analytical balance.
3. Hold the containers over water (but not touching it) in a desiccator for about a week.
4. Remove the containers from the desiccator and allow the surface to dry.
5. Weigh the containers, record the change in weight and examine the colour of the silica gel.
   - If the weight of the containers remains constant, then they are moisture proof and the seal is good.
   - If the weight of the containers increases and the silica gel has turned pale blue or pink, then they are of poor quality or the seal is leaking moisture.
6. Adjust the seal and repeat the test to confirm the quality of the containers.

A container's quality can also be tested by filling it with water and holding it over silica gel in a desiccator or in a ventilated oven at 40°C for one to two weeks. A change in the container's weight indicates poor quality or leakage in sealing.

How many seeds should be packed?

The number of seeds to be packed for storage will depend on the species being conserved and how often seeds will be removed for monitoring, distribution or regeneration. The FAO/IPGRI Genebank Standards (1994) recommend that for material which shows little morphological variation (genetically homogenous accessions), 3000 seeds are acceptable, but 4000 seeds are preferable to represent each accession. For materials showing large morphological variation (genetically heterogeneous accessions), an accession should consist of at least 4000 seeds, but 12 000 seeds are preferred.

In genebanks, it is easier to work in weights, but seed number can easily be converted from weights if the 100-seed or 1000-seed weight is known. For example, to determine the number of seeds in a sample for which the 100-seed weight is known:

$$\text{Number of seeds in the sample} = \frac{\text{Sample weight (g)} \times 100}{\text{Weight of 100 seeds (g)}}$$

Example:

Sample weight = 275 g
Weight of 100 seeds = 12.5 g
Total number of seeds in the sample = $\frac{275 \times 100}{12.5} = 2200$

How should seeds be packaged?

Packaging is best carried out in an air-conditioned room where the RH is controlled. It is important to ensure that seeds taken from the drying room are exposed to ambient air for the shortest time possible so that they do not re-absorb water.

1. Decide on the most suitable container for storing the seeds. Different types of containers can be used depending on the size and shape of the seeds, and the purpose of conservation (whether for base or active collections—see section 6.2).
2. Prepare and label the containers for each accession; computer-generated self-adhesive labels and barcodes[11] are now being used in many genebanks. Bar-coding ensures that information is accurate and that no mistakes occur during transcription. Prepare a label to include with the seeds inside the container. Labels should contain the following information:
   - Accession number
   - Genus and species
   - Container number
   - Weight of seeds
   - Date of storage
3. Weigh out each empty labelled container.
4. Fill the containers with the seeds and weigh again. Calculate the actual weight of the seeds.
5. Add the label and seal the container immediately to protect the seeds from high ambient RH.
6. Check the quality of each container after sealing it by making a visual examination to ensure that there is no leakage.
7. Any container found to be below standard should be replaced immediately.
8. Move the containers into the storage room.

[11] Bar-coding is a computerized coding system that uses a printed pattern of bars of varying widths to uniquely identify accessions. Bar-codes are read by optically scanning the printed pattern and using a computer program to decode the pattern. The data contained in a bar-code can vary: in its simplest form, it can be just an accession number, while in other cases, the bar-code can hold more elaborate passport and inventory details. Bar-coding provides enormous benefits to genebanks by allowing data capture that is faster and more accurate to minimize errors and facilitate inventory management.

9. Enter relevant data about each accession into the data file. A model for recording container and seed weight is shown in Table 6.1.

Table 6.1. Model table for recording information on seed packaging.

Date of packaging:			**Name of staff:**		
Accession number	**Type of container**	**Container number**	**Weight of container empty**	**Weight of container and seeds**	**Weight of seeds**
		1			
		2			
		3			

Reference samples (seed herbaria)

Seed herbaria are useful to check physical attributes of seeds without having to open sealed containers. Pack a small sample (five to ten seeds or pods of legumes, or 5 g of cereals) of the original seeds separately in a transparent re-sealable plastic envelope or glass vial to verify genetic integrity after regeneration and during seed transfer. The samples can be stored in a cabinet with closely spaced shelves. Ensure that seeds of the original sample are never completely exhausted so that they may serve as a reference for identification.

Some precautions

- Do not mix seeds harvested in different seasons as the quality and longevity of the samples can be different. Assign batch numbers (indicating season of harvest, site or field number and generation number) to differentiate the seed lots.
- Keep seeds from different seasons in separate containers or within the same container using separate cloth or re-sealable plastic bags if the container can accommodate them.
- Remember that containers removed from cold storage or freezers should be allowed to warm to room temperature before opening to avoid condensation of water on the seed surface. This may take several hours, especially for large seeds and those removed from sub-zero temperatures.
- The self-adhesive labels and ink used for labelling must be water resistant and very durable.

To ensure long-term conservation and continued availability of high-quality seeds for utilization, seeds packed in moisture-proof containers should be stored under controlled environmental conditions as described in the following section.

Further reading

FAO/IPGRI, 1994. Genebank standards. FAO and IPGRI, Rome.

6.2 Seed storage

What is seed storage?

Seed storage is the preservation of seeds under controlled environmental conditions that maintain seed viability for long periods.

The longevity of seeds depends on initial seed quality, moisture content and temperature during storage. In general, low moisture content and low temperature reduce the loss of seed viability. Different combinations of moisture content and temperature can be used to prolong seed viability during storage.

Types of storage

Two types of seed stores are used for conservation of genetic resources: those holding seed samples for long-term security—referred to as *base collections*—and those holding seed samples for immediate use—referred to as *active collections*. The temperature, RH, seed moisture content, containers and distribution arrangements of these stores vary.

Base collections

A base collection is a set of accessions in which each is distinct and as close as possible to the original sample in terms of genetic integrity. Normally, seeds are not distributed from base collections directly to users but are only used to regenerate active collections (FAO/IPGRI, 1994). Base collections are stored for long periods at below 0°C—usually at -18° to -20°C—to maintain seed viability.

Engels and Visser (2002) introduced the term 'most-original sample' (MOS) to qualify the samples in base collections. A MOS consists of seeds that have undergone the lowest number of regenerations since the material was acquired by the genebank; it may be a sub-sample of the original seed lot or a seed sample from the first regeneration cycle if the original seed lot required regeneration before storage.

Active collections

Active collections consist of accessions that are immediately available for distribution. These accessions are accessed frequently and maintained in conditions that ensure at least 65% viability for 10–20 years (FAO/IPGRI, 1994). Combinations of temperatures and moisture contents for storage of active collections that can assure viability above 65% for 10–20 years are given in Table 6.2. It is more practical to use a lower moisture content and store at a higher temperature to save on refrigeration costs. However, when drying

to the low moisture contents is not possible, storage with higher moisture but at a lower temperature can be considered.

Table 6.2. Suggested storage temperature and moisture content for active collections (source: Bioversity, unpublished).

Temperature (°C)	Storage characteristics	
	Poor (e.g. onion)	Good (e.g. barley)
	Moisture content (% wet basis)	
25	3	7
20	3.5	7.5
15	5.0	8.0
10	6.0	9.0
5	7.0	10.0
0	8.0	11.0

Organization of collections

The underlying principle of maintaining a base collection or MOS is that at least some seed of the original sample should be kept aside under the best possible conditions to ensure safe long-term survival. This may be achieved by keeping seeds for distribution physically separate (as active collections) from the original sample but there is no absolute requirement to do so. A genebank may opt to maintain one sample of each accession for both conservation (in a base collection) and utilization (in an active collection) as long as the maintenance cost is not too high. If the genebank maintains both base and active collections, it is more cost effective to store only those accessions in the active collection that are being used by breeders and other users. (For further information, see Engels and Visser, 2003.)

Type of storage facility

The two commonly available options for seed storage are *walk-in cold stores* and *freezers*. The choice depends on the number of accessions to be stored, seed size and storage temperatures selected. When collections are small and sub-zero temperatures are required, chest or upright freezers are a cheaper option for seed storage.

How is storage space organized?

The organization of storage space depends on the type of storage facility and the type of containers used in the genebank. In view of the cost of maintaining cold storage, the space should be optimized so that a maximum number of seed accessions can be stored.

Walk-in cold storage

If the genebank has a walk-in cold store, the best option is to use

moveable racks that maximize storage space. Each rack is divided into a number of shelves. The distance between each shelf will depend of the size of containers. Small containers or aluminium foil bags can be held in boxes or trays and placed on the shelves.

A coding system can help genebank staff to locate accessions readily for sample retrieval; coding can be computerized in a database or stock inventory system. For example, 'A010201' could be used to indicate the following location:

- Room number (if more than one storage room is being used): A
- Rack number: 01
- Shelf number: 02
- Tray/box number: 01

Chest or upright freezers

For genebanks using chest or upright freezers, containers that fit onto shelves or boxes holding individual small containers can be used to store accessions. As for cold storage, a coding system to help locating accession can be established, including freezer number, row number and box number.

Storing seed samples

Step 1: Check the number of seeds in the accession

1. Weigh the seeds of each accession. Convert the seed weights to numbers by using the 100-seed or 1000-seed weight as described in the previous section.
2. Verify if the sample contains more than the required number of seeds for a genetically homogenous sample (3000–4000 seeds) or a genetically heterogenous sample (4000–12 000 seeds).
3. If the sample contains less than the required amount, either proceed directly to regeneration or store temporarily in the genebank and regenerate at the earliest opportunity (see Chapter 8).

Step 2: Identify a location for storage

The next step is to determine the location inside the storage room or freezer in which the accession will be stored.

1. Check the inventory file to find the next available space for the accession.
2. Assign the space where the accession is to be placed. If the accession is stored in more than one container, keep them all together.

Step 3: Place seeds in store

1. Make a list of assigned spaces where each accession will be placed.
2. Place the containers in the storage room or freezer in their assigned locations.

Step 4: Enter data into the database

1. Enter the data regarding storage location, date and number of containers in the inventory file.

Safety duplication (security backup collection)

Safety duplication means a genetically identical sub-sample of the accession is stored at another location (preferably outside the country) to provide insurance against loss of material. Safety duplication includes both the duplication of material and its related information. Samples are prepared for safety duplication in the same way as the base collection:

- Seeds should be dried to a moisture content of 5±2% depending on species.
- Seeds should be clean and healthy.
- Percent germination should be greater than 85%.
- Seeds should be hermetically sealed in appropriate containers.

Sample size can be smaller, but it should be sufficient to conduct at least three regenerations (with the safety factor built in). To save time, samples for safety duplication may be prepared simultaneously when processing seeds for base collection.

Specific agreements should be made with the recipient institute for holding the duplicate of a collection. Ideally, duplicate collections should be held in the same conditions as base collections to ensure long-term survival, although several types of duplication are recognized:

- *Black box:* when the sole responsibility of the recipient genebank is to maintain the duplicates without handling them. Beyond providing the best possible storage conditions, the recipient institute has no further responsibility for the samples. It is the originator's responsibility to establish a viability monitoring scheme and regenerate the collection when necessary. If the storage conditions for the backup collection are the same as for base collection, loss of viability can be predicted from the results of base collection-monitoring. Following regeneration of the sample in the base collection, the originator also replaces the safety duplicate. For black-box duplication outside the country, special permission is required to export seeds without *phytosanitary certificates* from the originating country. Similarly, the phytosanitary authority in the destination country must permit the recipient to import seeds without the routine quarantine examination.
- *Base:* maintained under suitable condition for long-term storage and incorporated into the recipient collection.
- *Active:* when the duplicate collection is incorporated into the recipient's collection, and is therefore subject to regeneration, multiplication and distribution by the recipient.

Archive collection

Genebanks may choose to store germplasm samples that do not need to be represented in a base collection or distributed as an 'archive collection'. These samples are maintained under optimal conditions for long-term survival but with no further investment in monitoring and regeneration. Germplasm included to archive collection can be:

- experimental lines bound by intellectual property (IP) rights—samples can be held as black-box collections and returned upon request to the IP holder;
- germplasm that lies outside the genebank mandate—samples can be stored temporarily until another genebank with a relevant mandate is identified;
- accessions identified as duplicates following rationalization of an existing base collection; and
- accessions no longer needed in the collection following a reassessment of the genebank's mandate or material disbanded due to lack of funding.

Documentation

Proper documentation of seed packaging and storage procedures allows rapid accession of new samples; response to queries regarding conserved germplasm; and monitoring of the quality and quantity of stored material to carry out regeneration and distribution. Suggested descriptors include the following:

- Storage conditions/collection type
- Type of container, if this varies in the genebank
- Number of containers
- Total amount of seeds stored (by weight or number)
- Date of storage
- Location in genebank
- Minimum amount of seed allowed (base unit) for dissemination/regeneration
- Location of safety duplicate, if available

Further reading

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FAO/IPGRI, 1994. Genebank standards. FAO and IPGRI, Rome.

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7. Distribution

7. GERMPLASM DISTRIBUTION

What is germplasm distribution?

Germplasm distribution is the supply of representative samples of seed accessions from a genebank in response to requests from germplasm users. In general, seeds are distributed only from active collections (see Flowchart 7.1).

Why is germplasm distributed?

The purpose of conserving germplasm in a genebank is either to improve crop varieties through plant breeding and related research activities or to restore diversity lost on farms and in natural habitats in order to meet the needs of farmers and communities. This contributes directly to improving the livelihoods of poor people and to protecting the environment.

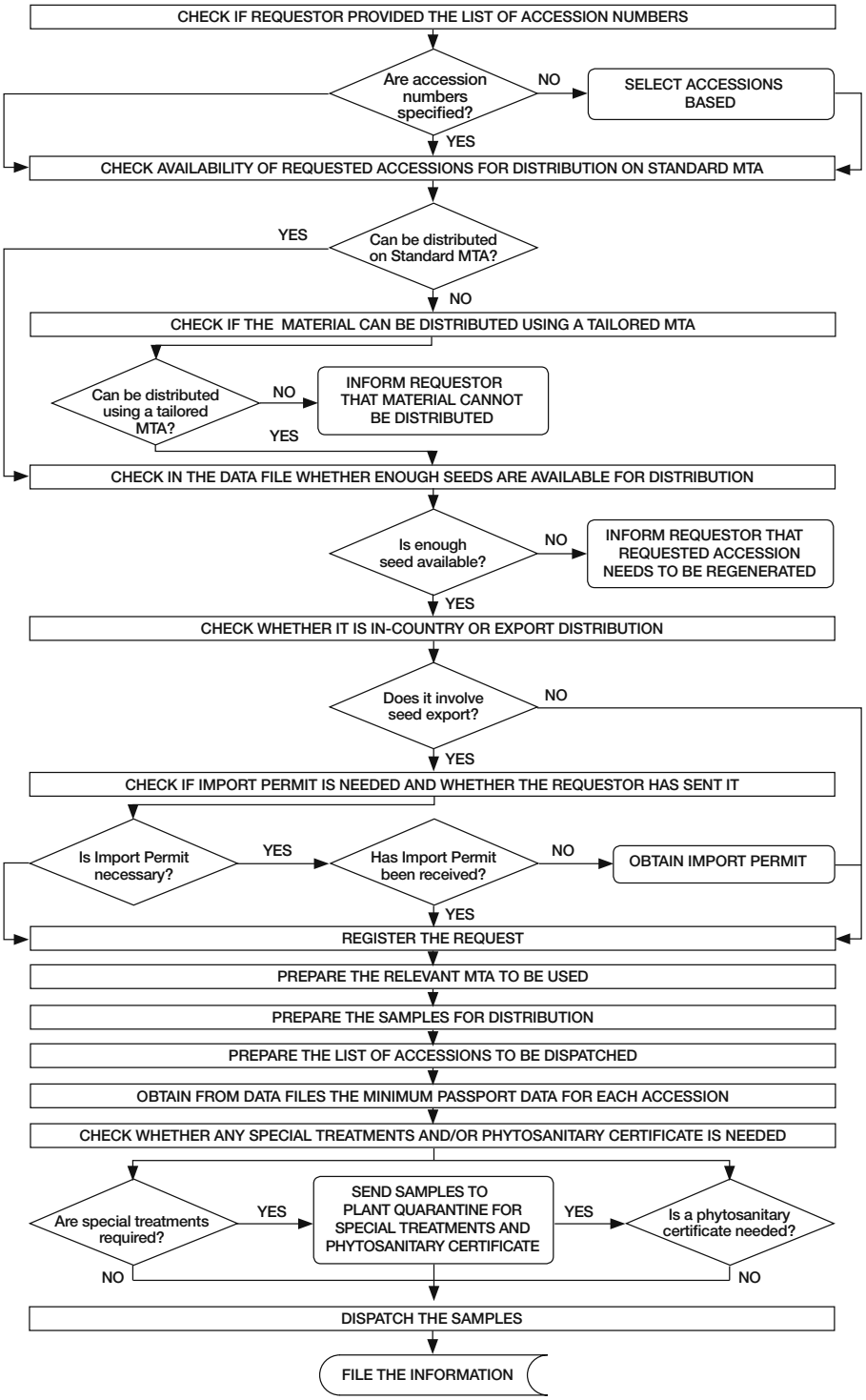
In the past, not enough emphasis was given to germplasm distribution. It is now widely recognized that the utilization of germplasm should drive its conservation. Genebanks must be more proactive in establishing links with germplasm users, breeders, researchers, farmers and other groups.

How should germplasm be distributed?

Germplasm should be distributed in a way that ensures it reaches its destination in good condition. Environmental conditions during transport can be detrimental to seed quality, so seeds should be carefully packed and distributed in sealed moisture-resistant envelopes for protection during transit (see below).

The scope and extent of distribution varies with each genebank. Germplasm may be distributed within or outside the country, depending on the genebank's mandate and whether its collection is national, regional or global.

Flowchart 7.1. Germplasm distribution.



Procedures for in-country seed distribution

Step 1: Decide whether the accession can be distributed

- Check the inventory database to see if the seed quantity in the genebank is sufficient for distribution.
- Distribute only if a minimum of four to six times the number of seeds required for one regeneration cycle remain in store after meeting the request. Some flexibility may be allowed in cases where the accession is rarely requested.
- When seed quantity is inadequate for distribution, inform the requester that the accessions cannot be supplied until after regeneration, and prepare the accessions for regeneration.
- Check the passport data to determine the material's status in relation to access and benefit-sharing under the International Treaty on PGRFA and other international agreements. If there are restrictions on distribution under the germplasm acquisition agreement (GAA) with the donor (see Annex I), inform the requester accordingly.

Step 2: Prepare the sample for distribution

If seeds are available for distribution:

1. Register the request by assigning a request number.
2. Prepare the list of accessions available for distribution.
3. Check the requirements for a material transfer agreement (MTA); if the material cannot be distributed under the SMTA, use a tailored MTA for the selected accessions (see Annex I for more information).
4. Prepare two sets of labels for the selected accessions and paste one of them on the envelopes (preferably of laminated aluminium foil) that will be used for distributing seeds to the requester.
5. Check the inventory file and note the location of the containers in the genebank.
6. Move the containers from the genebank into a dehumidified room the evening before distribution to allow them to warm to room temperature before opening. Ensure absolute accuracy in identification of accessions while drawing the seeds from the genebank.
7. Open the container and quickly draw the required amount of seeds into the labelled envelopes. Use random sampling so that a good representation of the accession is provided. It is suggested that 50–100 viable seeds should be distributed to fill each request, depending on the breeding system of the species (more for cross-pollinating and less for self-pollinating species).
8. Close the container immediately after removing the seeds for distribution to prevent uptake of moisture from ambient air.
9. For extra security, a second label may be placed inside the envelopes before packets are sealed.

10. Compare the list of accessions drawn from the genebank with the labels on the envelopes.

Step 3: Prepare the information list to accompany the seeds

1. Print the final list, including passport details such as accession number, alternate identity, source country, location and biological status, as well as characterization data used to verify accessions and any information solicited by the requester.
2. Prepare a cover letter.

Step 4: Dispatch the seeds

1. Pack the seed envelopes, cover letter, MTA and the seed list in a plastic bag and then in a strong envelope (if there are few samples) or a cardboard box (use filling material to avoid damage to seeds during transit). Label the envelope or box with the complete address of the requester. The MTA may be pasted on the outside of the envelope in cases where opening the container and using the seeds signifies agreement with the terms and conditions of access.
2. Include a reply form for the requester to complete and return to the genebank to acknowledge that seed samples have been received in good condition.
3. Send the seed parcels by the fastest means, such as by courier, to avoid delays and deterioration of seed quality during transit. If there is any concern that the materials could be lost during shipment, use registered mail or carry by hand if possible.
4. Record the shipment details in the distribution data file.
5. Update the seed inventory by deducting the weight or number of seeds supplied.

Germplasm seeds are valuable and should be packed carefully for dispatch. Packing should ensure safety of the seeds and prevent contamination by insects or pathogens during transit.

Distribution of germplasm outside the country

Follow the same procedure for selecting accessions and fulfilling the MTA requirement as described in steps 1 and 2 above. Additional requirements may be needed for distribution of germplasm across borders before moving to steps 3 and 4. These relate to compliance with phytosanitary regulations (see below) to avoid the danger of introducing pests and diseases into new areas.

How phytosanitary measures affect seed movement

Movement of any seeds can potentially spread pests.[12] There are many places around the world where this has already occurred with devastating effects. Recognizing this danger, all countries have

Consignments of germplasm infested with pests or without proper documentation will be refused entry or destroyed. Genebank staff need to be aware of the phytosanitary regulations governing trans-boundary germplasm movement.

[12] The International Plant Protection Convention (IPPC) defines pests as all harmful and potentially harmful biotic agents, from viroids to weeds.

phytosanitary measures to regulate the entry of plants, plant parts and their products. It is therefore essential to meet the national requirements of the importing country when moving seeds across international boundaries.

What are phytosanitary measures?

A phytosanitary measure is any legislation, regulation or procedure aimed at preventing the introduction or spread of quarantine pests, or limiting the economic impact of regulated non-quarantine pests. These measures are established by the importing country following a pest risk analysis according to international standards.

Official documentation required for seed export includes a *phytosanitary certificate* issued by the national plant protection organization or officially authorized institute of the exporting country, certifying that the shipment meets the phytosanitary regulations of the importing country. Phytosanitary certificates help to ensure that commodities are free of injurious plant pests following inspection in the country of origin by a member of that country's national plant protection organization. The certifying country usually charges a fee for each certificate.

Phytosanitary information for many countries may be found on the official website of the International Plant Protection Convention (IPPC) at www.ippc.int. National IPPC contact persons should be contacted when:

- determining the phytosanitary requirements for importation of seed; or
- applying for phytosanitary certification for seed export.

When preparing for seed distribution, observe the following guidelines:

- Check the final destination and the latest phytosanitary import requirements for the importing country (in many countries, regulations are changed frequently, so this needs to be completed before each shipment—see also 'post-entry quarantine' in Chapter 2).
- Ensure that the national plant protection organization in the exporting country supplies the appropriate documentation, such as an official phytosanitary certificate, that complies with the requirements of the importing country.
- Determine the procedures for obtaining a phytosanitary certificate in the country of export.
- Knowledge of the appropriate certifying authorities will ensure success at all stages.

Procedure for seed export

1. Prepare a list of accessions that are needed to fulfil the request.
2. Draw the seeds from the genebank as described for in-country distribution.
3. Apply for a phytosanitary certificate, available with the national plant protection organization or designated institute.
4. Send the application to the appropriate phytosanitary authority and arrange the necessary treatments and inspections for the issuance of a phytosanitary certificate.
5. Obtain additional declarations for special treatments as required by the importing country.

6. When the samples are ready for dispatch, prepare a cover letter and final list of accessions along with passport data, characterization data and other information as described in step 3 above. Any accessions detained at quarantine should be removed from the final list.
7. Ship the seeds to the consignee[13] along with the phytosanitary certificate, any other declarations needed, the MTA and the cover letter.
8. Comply with any additional requirements such as obtaining a plant *import permit* or CITES permit for endangered species (see Annex I) before shipping the seeds.
9. Record the shipment details in the distribution data file and update the seed inventory by deducting the weight or number of seeds supplied.

If mandatory treatments are prescribed as a phytosanitary measure, or endorsements are required, they should be provided by a government authority exactly as requested. For example, fumigation may be requested, samples may need to be dipped in an insecticide or fungicide, or a hot-water treatment may be required by the importing country. The treatments should be detailed on the phytosanitary certificate along with any other endorsements requested by the importing country. If no treatments are requested, none should be administered since these treatments can mask symptoms of seed-borne pathogens and interfere with laboratory tests. Pre-treatment prior to entry against the importing country's specifications could seriously jeopardize the shipment. Where germplasm samples are to be sent to more than one country, it is necessary to obtain a phytosanitary certificate for each destination. Two copies of the phytosanitary certificate must be obtained and the original should accompany the consignment. Any uncertified alterations or erasures will render the phytosanitary certificate invalid.

As cultivation of transgenic or genetically modified crops expands, many countries now require a certificate from an independent accredited entity confirming that the consignment is free from GMOs (see also Annex I).

Feedback on germplasm utilization

Obtain feedback on the usefulness of germplasm supplied to users at half-yearly intervals. This will help to identify deficiencies in service and remain informed about any new traits or sources of resistance identified.

[13] Phytosanitary regulations in some countries stipulate that consignments should be addressed directly to phytosanitary authorities and not to the consignee, and shipped through specific ports of entry.

Documentation

It is important that genebanks keep records of germplasm recipients, the number of samples sent, accession details and the purpose for which requests are made in order to track the use and assess the impact of distributed germplasm. It is recommended that the information be maintained in two files with a 'common link' field. Assigning a 'reference number' while registering a seed request can serve as a link field for the two files. Distribution descriptors can also be organized into two files, namely:

- a master file with details of the consignee, number of accessions sent, etc.; and
- an accession-details file containing information about the material.

The following descriptors are suggested for the distribution.

Master file

- Distribution reference number
- Recipient's address
- Date requested
- Date of supply
- Total number of accessions distributed
- Purpose of request
- Phytosanitary certificate (where applicable)
- Export permit number (where applicable)
- Recipient's *import permit* number (where applicable)

Accession details file

- Distribution reference number
- Accession number
- Amount of seeds distributed
- Designation status of materials in trust or under the International Treaty

8. GERMPLASM MONITORING AND REGENERATION

8.1 Germplasm monitoring

What is monitoring?

Monitoring is the regular checking of quality (viability) and quantity (number or weight) of germplasm accessions stored in a genebank. The objective of monitoring is to determine whether regeneration or multiplication of an accession is required.

Why should accessions be monitored?

Accessions are monitored for two main reasons:

- The viability of seeds stored in the genebank decreases during storage; it is important to monitor viability of accessions to ensure that they do not lose their capacity to produce viable plants when needed.
- The removal of seeds for distribution and germination testing results in a decrease of seed quantity over time.

To avoid excessive deterioration of seed quality or quantity, genebank accessions should be monitored both for viability and seed quantity during storage.

How frequently should accessions be monitored?

Seed quantity by number or weight should be monitored every time seeds are distributed from the genebank. This facilitates immediate identification of accessions with insufficient quantities of seeds for further conservation. Viability-monitoring tests should be conducted regularly. The monitoring interval depends on the species, storage environment (seed moisture content and temperature) and viability at the beginning of storage.

- The FAO/IPGRI Genebank Standards (1994) recommend that the first monitoring test should be conducted after ten years for seeds stored in base collections under preferred conditions (-18°C) with high initial viability (>90% germination).

- Seeds of species known to have poor longevity, including most oily crops and accessions with relatively low initial viability (85–90% germination) in base collections, as well as all seeds stored in active collections under preferred conditions (see Table 6.2), should be monitored for viability after five years.
- The interval between subsequent tests should be based on experience, and may be adjusted up or down depending on the extent of viability loss observed during the first monitoring test (see Table 8.1).

Remember to update the seed quantity in the inventory database, deducting the number of seeds drawn for the germination test. Also, update the germination data in the inventory database after the final result is obtained.

Viability monitoring

Viability is monitored by conducting a germination test on a fixed sample or by sequential germination (see Flowchart 8.1).

Fixed sample size germination test

For the fixed sample size germination test, it is recommended to use a minimum of 200 seeds (as two replicates of 100 seeds). If the quantity is limited, 50–100 seeds can be tested in two replications.

1. Identify and list all accessions that require testing, and schedule the tests on a weekly or monthly basis (depending on availability of space in germinators and human resources).
2. Locate the containers in storage from the inventory.
3. Remove the containers from storage and leave them overnight at room temperature to warm.
4. Open each container, draw a sample of seeds for the test and immediately close the containers.
5. Conduct the germination tests using the methods and conditions described in Chapter 5.
6. Calculate the mean percentage germination from the results of the two replicates. Repeat the germination test if the difference between the two replicates exceeds 10% or the maximum tolerance limits at 2.5% probability (see Ellis et al., 1985).
   - If the percentage germination is above 85% of the initial germination percentage, continue to store the accession. Fix the date for next test depending on the current percentage germination (see Table 8.1).
   - If the mean germination is below 85% of the initial germination percentage, schedule the accession for regeneration (see Table 8.2).

Sequential germination tests

The sequential germination test uses fewer seeds per replicate than the standard germination test. Otherwise, the methods and conditions for germination are the same as described for the fixed sample size germination test.

Flowchart 8.1. Viability monitoring.

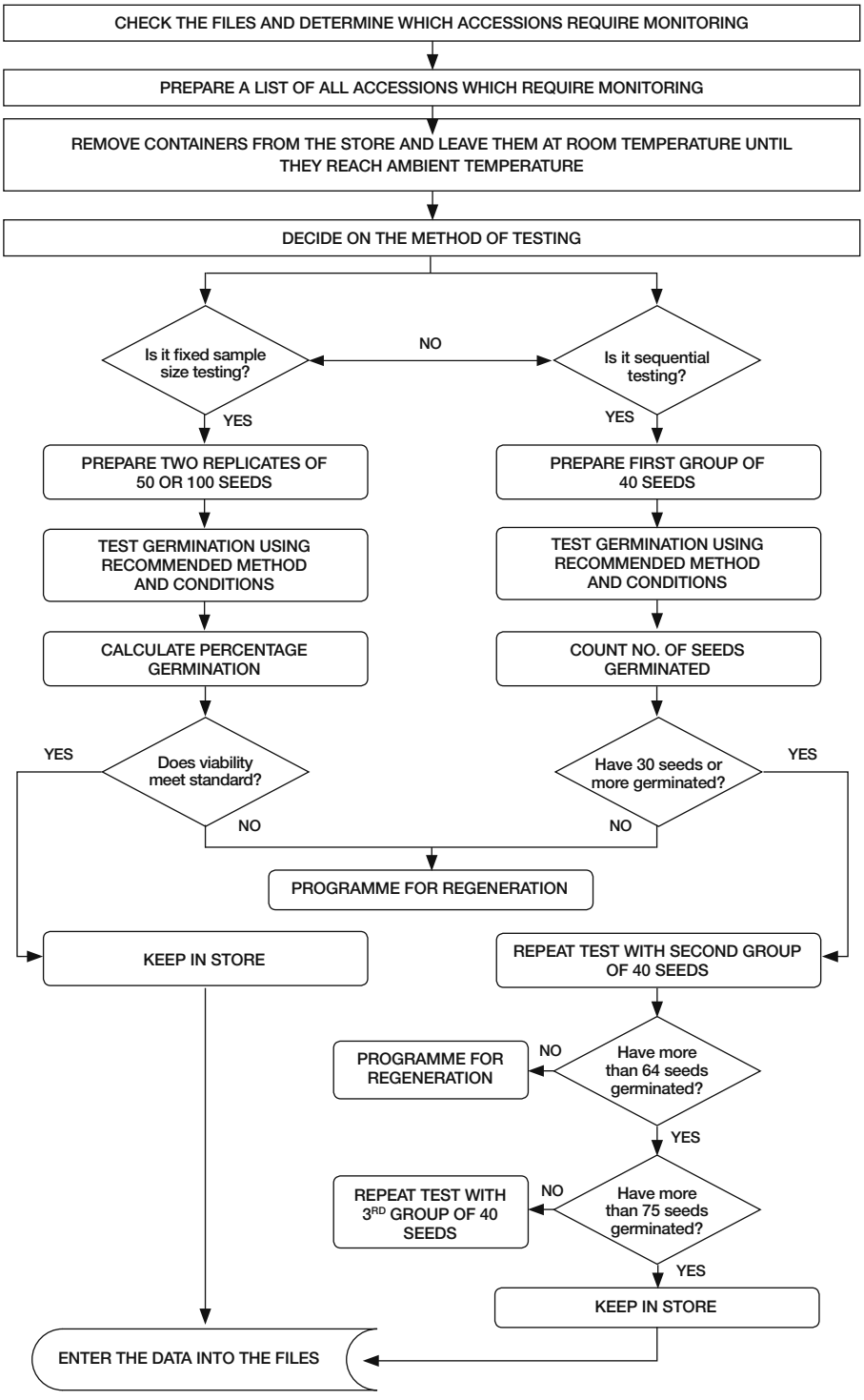


Table 8.1. Suggested interval for monitoring germination of active or base collections in oily and non-oily seeds.

Present level of germination (%)	Monitoring interval (years)			
	Active collection (4–5ºC)		Base collection (-20ºC)	
	Non-oily seeds	Oily seeds	Non-oily seeds	Oily seeds
<80	3	1	5	2
80–85	5	3	10	5
85–95	8	5	15	8
>95	12	8	20	12

Table 8.2. Threshold germination percentages for regeneration of accessions.

Initial germination	Regenerate if percentage germination after monitoring is below
100	85
99	84
98	83
97	82
96	82
95	81
94	80
93	79
92	78
91	77
90	77
89	76
88	75
87	74
86	73
85	72

The number of seeds required for each replicate may vary, but it is recommended to use at least 40 seeds per replicate.

1. Conduct the germination test according to the methods described in Chapter 5 using (for example) 40 seeds
2. Count the number of seeds germinated after the prescribed period of testing.
3. Compare the results with the number germinated in Table 8.3, paying attention to the line with the value of 40 in the first column (number of seeds tested).
   - If the number of seeds germinated is 29 or less, the accession requires regeneration.
   - If the number of seeds germinated is more than 29, then the test must be repeated with another sample of 40 seeds exactly as described above.

Table 8.3. Sequential germination test plan for 85% regeneration standard when testing seeds for germination in groups of 40.†

Number of seeds tested	Regenerate if the number of seeds germinated is less than or equal to	Repeat test if number of germinated seeds is in the range of	Store if number of seeds germinated is more than or equal to
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	340	-	341

† When 400 seeds have been tested, the test can be terminated because enough tests have been conducted for an informed decision to be made.

It is important to use the same number of seeds when repeating the test so that the different samples can be treated as replicates.

4. Count the number of seeds germinated in the second test and add this number to the result of the first test.
5. Compare the results of the test with the number germinated in Table 8.3, following the line with the value equal to the total number of seeds used for all tests (80 seeds) in the first column (number of seeds tested).
   - If the number of seeds germinated is 64 or less, the accession must be regenerated.
   - If the number germinated is above 75, the accession can be continued in storage.
   - If the number germinated is between 65 and 75, the accession must be tested again with another sample of 40 seeds and the results compared with the value equal to the total number of seeds used in all tests (120 seeds) in Table 8.3.
6. Continue the test in this way until a decision can be made regarding regeneration or continued storage, or until the test is repeated ten times.

For more information on test plans for other group sizes (20, 25, 50 or 100 seeds) and regeneration standards between 65% and 80%, refer to Ellis et al. (1985). The sequential test is only necessary when seed numbers are limited. Small-seeded crops like finger millet

normally have adequate numbers of seeds to use the fixed sample size method.

Monitoring seed quantity

Seed quantity can be monitored by checking the inventory data file. This is best achieved through a computer-based genebank documentation system.

1. Record the weight of the seeds initially transferred to the genebank.
2. Record all subsequent seed withdrawals for distribution, regeneration and germination-testing.
3. Update documentation of seed stocks *immediately,* adjusting the total after all seed withdrawals.
4. Prepare a list of accessions where the number of seeds in storage has fallen below the critical level (usually the number required for at least three regenerations).

Germplasm accessions identified with low viability or inadequate quantity during the course of monitoring should be regenerated as soon as possible using the method described in the following section.

Documentation

Monitoring is a crucial activity in genebank management as it helps to provide information on seed stocks that are becoming low, accessions that need viability testing and those that require regeneration. Without proper documentation of the data from preceding genebank activities, effective monitoring is difficult to carry out.

8.2 Germplasm regeneration

What is germplasm regeneration?

Regeneration is the renewal of germplasm accessions by sowing and harvesting seeds that possess the same characteristics as the original sample. Germplasm regeneration is the most critical operation in genebank management.

Why is regeneration critical in genebank management?

Germplasm regeneration involves risks to the genetic integrity of germplasm accessions due to selection pressures, out-crossing, mechanical mixtures and other factors. The risk of genetic integrity loss is usually high when regenerating genetically heterogeneous germplasm accessions. Germplasm regeneration is also very expensive.

Why should germplasm be regenerated?

Germplasm is regenerated for the following purposes:

1. Initial seed increase

In new collections or materials received as donations, the quantity of seeds received by the genebank is often insufficient for direct conservation. Seeds may also be of poor quality due to low viability or infection. All these materials require regeneration. Newly acquired germplasm of foreign origin may need to be initially regenerated under containment or in an isolation area under the supervision of the national phytosanitary authority as described in Chapter 2.

2. Replenishing seed stocks in active and base collections

Increase seed stocks of accessions that have:

- low viability identified during periodic monitoring; or
- insufficient stocks for distribution or conservation.
- Active collections should be regenerated from original seeds in a base collection; this is particularly important for out-breeding species. Using seeds from an active collection *for up to three regeneration cycles* before returning to the original seeds (base collection) is also acceptable (FAO/IPGRI 1994).
- Base collections should normally be regenerated using the residual seed from the same sample.

3. Meeting special requirements

There may be special requirements for regeneration of accessions with special traits that breeders and researchers use frequently—such as high-yielding, pest- and disease-resistant accessions and genetic stocks — or if there are insufficient seeds for safety duplication and repatriation.

Consider the following factors when regenerating germplasm accessions:

- suitability of environment to minimize natural selection;
- special requirements, if any, to break dormancy and stimulate germination (such as scarification);
- correct spacing for optimum seed set; and
- breeding system of the plant and need for controlled pollination or isolation.

Procedures for regeneration

- If possible, regenerate germplasm in the ecological region of its origin. Alternatively, seek an environment that does not select some genotypes in preference to others in a population.
- If no suitable site is found, seek collaboration with an institute that can provide a suitable site or regenerate in a controlled environment such as a growth room.
- Examine the biotic environment in the context of prior information about the plants and past experience—an inappropriate biotic

Genebanks should adopt a high regeneration standard (such as percentage germination to which an accession conserved in a genebank is allowed to reach before regenerating) to avoid genetic shifts resulting from natural selection of seeds of greater longevity in genetically heterogeneous accessions. The FAO/IPGRI Genebank Standards (1994) recommend that the initial germination value should exceed 85% for most seeds and regeneration should also be undertaken when viability falls below 85% of the initial value. Regeneration should be undertaken when the number of seeds in a base collection falls below the number required for at least three cycles of regeneration.

environment can be detrimental to plants, seed quality and the genetic integrity of an accession.

Selection of accessions

- Regeneration of accessions that have inadequate quality (low viability) should take priority over that of accessions with inadequate numbers of seeds.
- Regenerating accessions in base collections should take priority over regenerating those in active collections.

Preparation of regeneration plots

Soil

- The regeneration plot should be as uniform as possible.
- The field should have good drainage.
- Consider the need for soil analysis and apply treatments appropriate for the crop and site (fertilizers, lime, irrigation or solarization).

Solarization

Solarization consists of heating soil by covering it with polyethylene sheets during the hot summer in the tropics to control soil-borne diseases; it is conducted for at least six weeks during the hottest part of the year.

1. Thoroughly cultivate the land and level it to minimize protrusions.
2. Give 50 mm irrigation before laying the polythene sheets.
3. Use clear transparent polythene sheets, 1–2 mm thick.
4. Insert two edges of each polythene sheet in the furrows and bury the edges in the soil tightly.
5. Place weights to prevent flapping and tearing of polythene sheets in the wind.
6. When planting, leave a buffer zone of at least 0.5 m around the edges of the solarized area for dilution of heat near edges.
7. Do not allow irrigation water to flow in from other areas after solarization and during crop growth.

Weeds and soil-borne pests and diseases

- Identify weeds, pests and pathogens by inspection and prior experience.
- Consider reducing such problems during preparation of regeneration plots by applying the following treatments:
  - herbicide spray;
  - sterilizing soil;
  - ploughing to encourage germination of weeds, followed by herbicide spray; and
  - deep ploughing to kill emerging weeds.

Cleanliness

- Keep the plots absolutely clean from alien seeds and plants.
- Consider the risk of contamination with alien pollen and take appropriate measures during plot preparation, and by inter-cultivation and hand-weeding.
- Ensure that the method of plot preparation is appropriate for the chosen method of establishing plants (for example, ridges and flat beds).
- Prepare the regeneration plot considering:
  - the number of accessions to be regenerated;
  - the number of plants per accession;
  - spacing between rows and between plants; and
  - mechanical access for weeding.
- The method of preparation depends on:
  - soil structure;
  - the species to be sown or transplanted; and
  - the need for plant supports, in the case of climbers.

Preparation of seed

1. Dry, thresh and clean the seeds if the samples are newly acquired.
2. For those in storage:
   a. identify the candidate accessions that require regeneration;
   b. remove the containers from the genebank and allow them to warm up; and
   c. draw seed samples, keeping in mind the minimum sample size required for regeneration and the current level of germination.
3. Ensure absolute accuracy in identifying accessions while drawing the seeds from the genebank, packaging, and labelling seed samples. To minimize errors, it is the use of computer-based information management systems to generate labels is suggested.

The minimum number of seeds for regeneration can be estimated from the standard sample size used for regeneration and the sample viability according to the following equation:

Number of seeds required for regeneration = Desired plant population for regeneration/(Germination%[14] x Expected field establishment %[15]).

[14] Germination and field establishment percentage are expressed as decimals: 95% is expressed as 0.95.

[15] Plant establishment is generally 5% less than the germination percentage in poor conditions and 1% less in good conditions.

Example:

Desired plant population = 150
Percentage germination = 85
Expected field establishment = 80

$$\text{Number of seeds for plating} = \frac{150}{0.85 \times 0.80} = 220 \text{ seeds}$$

Seed pre-treatments

Specific pre-treatment may be necessary to improve seed germination and establishment. If the seeds are very dry (moisture content <8%), raise the moisture content by humidification as described in Chapter 5.

- Break dormancy for species or accessions (using stratification, scarification, etc.).
- Apply proprietary seed dressings to reduce disease and insect damage.
- Inoculate with appropriate symbionts (*Rhizobium* treatment for legumes).
- For accessions with limited seeds, pre-germinate in controlled conditions and transplant the seedlings into pots with sterilized soil and grow them in a screenhouse under close supervision.

Meiosis and anthesis are sensitive stages during plant development. Care must be taken to avoid any stresses such as high temperature and drought.

Sowing and crop management

Crop management for regeneration differs from normal commercial practices where interplant variation is not of primary consideration.
To avoid large losses of alleles and maximize seed yield:

- use 100 or more plants in genetically heterogeneous accessions;
- take special note of the day-length requirements for the species or they may not flower;
- provide suitable conditions for growth to trigger abundant flowering;
- eliminate competition by weeding alien plants; and
- ensure a stable source of water from irrigation if necessary.

Sowing date

- Sow at an optimum time so that maturity and harvesting coincide with the most favourable weather conditions.
- If there is much variation between accessions in flowering time, sort by early and late maturity based on previous documentation and adjust the planting dates so that all accessions mature in a uniform environment.
- Planting on the basis of maturity makes crop management and harvesting convenient.

- Sow in uniformly spaced rows and with uniform spacing between plants within rows.
- Avoid competition for light and nutrients by using wide spacing.
- Ensure complete control of pathogens and pests using standard plant-protection measures.
- Thinning should normally not be undertaken—if required, thin plants at random.
- Ensure continued absence of alien plants in the vicinity throughout the regeneration cycle by hand-weeding or using inter-cultivation.

Irrigation

- Irrigate the field when necessary.
- Never subject the crop to water stress.
- Ensure adequate drainage and no water-logging.

Regular inspection of plants is mandatory to achieve these objectives.

Verifying accession identity

- Accession identity should be verified while the plants are growing by comparing:
  - morphological data in the documentation system; or
  - reference material such as original herbarium specimens or seed.
- Roguing must be undertaken with caution and only when it is absolutely clear that the rogue plants are genuine mixtures of other accessions or varieties.
- When materials are grown in rows, plants growing off-row may be eliminated.

Pollination biology

Unless the species is an obligate inbreeder, appropriate pollination control should be implemented. A compendium of breeding mechanisms can be found at www.bioversityinternational.org/Themes/Genebanks/Species_Compendium/default.asp.

For out-breeders, elimination of alien pollen can be achieved through:

- spatial isolation (this is not practical when dealing with large number of accessions of the same species but very useful for dealing with a limited number of accessions of many species);
- temporal isolation;
- natural or artificial barriers—growing accessions in standing crops of tall-growing species like sunflower and hemp; and
- bagging selected inflorescences with pollen-proof or pollinator-proof bags made of linen or paper and erecting temporary pollen-proof or pollinator-proof nets around plots. Supplemental hand-pollination is sometimes required to improve seed set.

Insect-pollinated crops may be grown in net or nylon-screened cages with specially designed hives for insect pollinators like bees; one accession of each crop species may be planted in each cage. Insect pollinators are released inside the cage at the time of flowering. Supplemental hand-pollination may be necessary to improve seed set (such as in wild species of tomato and eggplant). Isolation cages can be expensive and shading may affect plant growth. An effective solution could be bagging and controlled pollination by hand. If plants flower during or at the end of the wet season, however, pollination bags may be damaged by rain. Excess moisture and humidity in the bags around the flowers can also lead to increased infection with bacteria and fungi. In wet or humid conditions, it is best to tag the flowers and remove the bag as soon as pollination is complete so that the fruits may develop under normal field conditions.

Harvesting and post-harvest management

- Harvest at optimum maturity (after the seeds have reached the point of physiological maturity):
  - when maximum number of seeds are ripe;
  - when seeds become tolerant to desiccation and can be threshed without mechanical injuries;
  - before deterioration sets in; and
  - before natural dispersal occurs.
- Stagger the harvest if there are differences in maturity of the accessions.
- Harvest individual plants within an accession when there are differences in flowering and maturity between plants.
- Mix an *equal proportion of seeds* from different mother plants to avoid maternal effects.
- Bags holding harvested seeds or heads should be made of porous material enabling good air circulation for drying.
- Options for harvesting depend on the crop:
  - Harvest plants individually, preferably by hand. If machine-harvesting, use purpose-built machinery because commercial machinery cannot be cleaned adequately between regeneration plots.
  - Harvest infructescences individually by hand.
- Initiate seed drying immediately after harvesting to prevent seed deterioration.
- If seeds cannot be processed quickly, they should be placed in a temporary holding area in a controlled environment such as an air-conditioned room.

Documentation

Regeneration is performed as a result of information generated by seed monitoring. As regeneration methods vary according to

species, the types of descriptors used to record information also vary. The following descriptors will help in documenting the data:

- Regeneration site
- Collaborator (where applicable)
- Plot reference
- Sowing date
- Germination in the field
- Number of plants established
- Days from sowing to flowering
- Breeding system
- Pollination control method used
- Harvest date
- Number of plants harvested
- Quantity of seeds harvested

Further reading

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Table 8.4. Reproductive behaviour and pollination control mechanisms for regeneration of important crops.

Crop	Species	Pollination behaviour (crossing rate)	Pollination mechanism	Method of regeneration
Alfalfa (lucerne)	*Medicago sativa*	Mainly CP (84-94%)	Insects (tripping)	Isolation; screened cages with pollinators
Amaranth	*Amaranthus* spp.	CP	Wind	Isolation; bagging
Barley	*Hordeum vulgare*	SP		
Black gram	*Vigna mungo*	SP		
Bottle guard	*Lagenaria siceraria*	CP; monoecious	Insects	Bagging and hand-pollination
Brown mustard	*Brassica juncea*	Mainly SP (4-14% cross-pollination)	Insects	Bagging
Buck wheat	*Fagopyrum esculentum*	CP; self-incompatible	Wind	Bagging and hand-pollination
Buffel grass	*Cenchrus ciliaris*	CP	Wind	Isolation; bagging
Cabbage	*Brassica oleracea* var. *capitata*	CP	Insects	Screened cages with pollinators
Carrot	*Daucus carota*	CP; protandrous	Insects	Screened cages with pollinators
Castor bean	*Ricinus communis*	CP; monoecious	Wind	Bagging and hand pollination
Cauliflower	*Brassica oleracea* var. *botrytis*	Mainly CP	Insects	Bagging
Chickpea	*Cicer arietinum*	SP		
Chicory	*Cichorium intybus*	CP; strongly self-incompatible	Insects	Spatial isolation; Bagging; insect-proof cages
Common bean	*Phaseolus vulgaris*	Mainly SP; cross-pollination 8-20%	Insects	Isolation; insect-proof cages; bagging
Cotton	*Gossypium* spp.	Mainly SP; natural outcrossing 10-50%	Insects	Bagging; insect-proof cages
Cowpea	*Vigna unguiculata*	Mainly SP		
Crotalaria	*Crotalaria juncea*	Mainly SP		
Cucumber	*Cucumis sativus*	CP; monoecious	Insects	Bagging and hand pollination
Eggplant	*Solanum melongena*	Partially SP; natural outcrossing up to 48%	Insects	Isolation; bagging
Faba bean	*Vicia faba*	Mainly SP; 4-8% outcrossing	Insects	Isolation; bagging
Finger millet	*Eleusine corocana*	SP		
Foxtail millet	*Setaria italica*	SP		
Grass pea	*Lathyrus sativus*	SP; significant levels of CP can occur		Bagging
Hyacinth bean	*Lablab purpureus*			
Lentil	*Lens culinaris*	SP		
Lettuce	*Lactuca sativa*	Mainly SP; natural outcrossing 1-6%	Insects	Bagging; insect-proof cages
Lima bean	*Phaseolus lunatus*	Mainly SP (up to 18% natural crossing)	Insects	Isolation
Linseed (flax)	*Linum usitatissimum*	Normally SP; natural crossing up to 12%	Insects	Isolation; bagging
Lupin	*Lupinus* spp.	Mainly SP; some CP can occur	Insects	Isolation; insect-proof cages or bagging

Crop	Species	Pollination behaviour (crossing rate)	Pollination mechanism	Method of regeneration
Maize	*Zea mays*	CP; monoecious	Wind	Bagging ear and hand pollination with pollen pool
Mung bean	*Vigna radiata*	SP		
Oat	*Avena sativa*	SP		
Okra	*Abelmoschus esculentus*	Partially SP; natural outcrossing 4-19%	Insects	Isolation; insect-proof cages or bagging
Onion	*Allium cepa*	Mainly CP; protandrous	Insects	Screened cages with pollinators
Pea	*Pisum sativum*	Mainly SP		
Peanut	*Arachis hypogaea*	SP		
Pearl millet	*Pennisetum glaucum*	Mainly CP; protogynous	Wind	Bagging; hand-cross with pollen pool
Pepper, chilli	*Capsicum annuum*	Often CP; heterostyly	Insects	Bagging
Pigeonpea	*Cajanus cajan*	Normally SP; natural outcrossing 5-40%	Insects	Isolation; bagging; insect-proof cages
Potato	*Solanum tuberosum*	Mainly CP	Insects	Isolation; bagging
Pumpkin	*Cucurbita moschata*	CP; monoecious	Insects	Bagging and hand pollination
Radish	*Raphanus sativus*	CP; strongly self-incompatible	Insects	Screened cages with pollinators
Red clover	*Trifolium pratense*	CP; strongly self-incompatible	Insects	Screened cages with pollinators
Rice	*Oryza sativa*	SP		
Rye	*Secale cereale*	CP; strongly self-incompatible	Wind	Bagging and hand-pollination with pollen pool
Rye grass	*Lolium perenne*	CP	Wind	Bagging
Safflower	*Carthamus tinctorius*	SP		
Sesame	*Sesamum indicum*	Mainly SP; cross pollination up to 5%	Insects	
Sorghum	*Sorghum bicolor*	Mainly SP; cross-pollination up to 1-50%	Wind	Isolation; bagging
Soya bean	*Glycine max*	SP		
Spinach	*Spinacea oleracea*	CP; dioecious	Wind	Spatial isolation
Strawberry	*Fragaria ananassa*	Mainly CP	Insects	Insect-proof cages
Sugar beet	*Beta vulgaris*	CP; self-incompatible	Wind, Insects	Spatial isolation, Screened cages with pollinators
Sunflower	*Helianthus annuus*	Partially CP; protandrous	Insects	Bagging and hand pollination
Sweet clover	*Melilotus albus*	SP		
Tobacco	*Nicotiana tabacum*	SP		
Tomato	*Lycopersicon esculentum*	Normally SP; some species self-incompatible with moderate to high CP		
Triticale	*Triticosecale*	CP	Wind	Isolation; bagging
Vetch	*Vicia sativa*	SP		
Watermelon	*Citrullus lanatus*	CP; monoecious	Insects	Bagging and hand pollination
Wheat	*Triticum aestivum*	SP		

SP= Self-pollinating; CP= Cross-pollinating

ANNEX I

International policies and frameworks influencing access to and exchange of germplasm

Germplasm assemblage and distribution essentially involve movement of seeds across locations or regions. In assembling germplasm, genebanks acquire or import material from germplasm collectors or other suppliers from within and outside the country. Distribution involves exporting seed samples to users worldwide. In addition to the phytosanitary regulations described in Chapters 2 and 7, the following international policies, frameworks and agreements influence the access and exchange of germplasm.

Convention on Biological Diversity (CBD)

The Convention on Biological Diversity (CBD), which came into force in December 1993, provided a legal framework for the conservation and sustainable use of plant genetic resources. Prior to the CBD, genetic resources were considered to be the common heritage of humankind and were freely available for use without restrictions. The CBD affirmed national sovereignty over genetic resources; in Article 15, it provided guidelines for access and use, including fair and equitable sharing of benefits from resource utilization (www.biodiv.org/convention/articles.asp).

International Code of Conduct for Plant Germplasm Collecting and Transfer

The International Code, adopted by the FAO Conference in 1993, provides a general framework for collecting and transfer of germplasm. It sets out the minimum responsibilities of collectors and curators regarding germplasm collecting and transfer. Although a voluntary instrument, the code is compatible with the CBD and serves as a reference for countries to establish their own regulations for germplasm collection and exchange (www.fao.org/ag/agp/agps/pgr/icc/icce.htm).

Germplasm Acquisition Agreements

Article 15 of CBD stipulates that access to genetic resources shall be on *mutually agreed terms* and subject to *prior informed consent.* Prior informed consent means that the providing country can grant or refuse access to germplasm following a request from the applicant. Access is on mutually agreed terms when both the supplier and recipient agree. This usually implies a contractual arrangement executed on a bilateral basis, which often takes the form of germplasm acquisition agreement (GAA) setting out the terms on which the genetic material is acquired and transferred.

International Treaty and Multilateral System for Access and Benefit Sharing

In 2001, the FAO Conference adopted the International Treaty on PGRFA, recognizing that: (i) agriculture in all countries depends largely on PGRFA that originated elsewhere; (ii) future advances in crop improvement require continued access to a wide genetic base without major restrictions; and (iii) a purely bilateral approach to access and benefit-sharing is not well suited to genetic resources of major food crops. The Treaty creates a Multilateral System for Access and Benefit Sharing covering 64 major crops and forages, and provides for facilitated access to genetic resources in the Multilateral System. The contracting parties are obliged to provide access for the purposes of food and agriculture research, breeding, and training when:

- they are requested to do so by another party, a legal entity under the jurisdiction of a party, or by an international institute that has signed an agreement with the governing body; and
- PGRFA have been acquired under these terms.

By the terms of the Treaty, countries agree that prior informed consent is not required for access to a defined category of PGRFA, but that a set of mutually agreed-upon terms will apply. The standard material transfer agreement (SMTA) enables access to plant genetic resources and establishes benefit-sharing based on royalties levied on commercial products that use material obtained through the multilateral system. For more details, see www.fao.org/ag/cgrfa/itpgr.htm.

CITES Permits

The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) is an international agreement between national governments that helps member countries control and monitor protected plant and animal populations. CITES regulates trade and exchange through permits and certificates. When importing samples of a species that is listed in the appendixes of CITES, a CITES import permit from the importing country and a CITES export permit from the country of origin must be obtained.

The species covered by CITES are listed in three appendixes according to the degree of protection they need.

1. Appendix I lists species that are the most endangered.
2. Appendix II lists species that are not currently threatened but face extinction unless trade is closely controlled.
3. Appendix III is a list of species for which the cooperation of other countries is needed to prevent unsustainable or illegal exploitation.

Seeds of Appendix II plants and seeds of artificially propagated hybrids of Appendix I plants are exempt from CITES controls.

Plants grown from exempt seeds are protected, however, and require CITES permits for import and export. The CITES-listed species database, including the three appendixes and national focal points for permits and certificates, is available at www.cites.org.

Genetically modified organisms (GMOs)

Several countries have established regulatory frameworks for importing and handling GMOs. These are largely based on the Cartagena Protocol on Biosafety (CPB) which came into force in September 2003 (see www.biodiv.org/biosafety/default.aspx). National biosafety authorities, in collaboration with phytosanitary authorities, are responsible for issuing import permits, conducting risk assessments and enforcing biosafety guidelines. Researchers wishing to import GMOs must submit an application providing: details of the genetic material to be introduced; accompanying information on research and testing of the GMOs in question; and a plan for safety measures to be followed during introduction. Approval is issued if the national authority determines that the GMO or GMO product poses no risk to the environment, biological diversity or human health. Prior to export of GMOs, authorization from the exporting country's biosafety authority is also required. No authorization for export will be given if the exporting country bans the GMO or GMO product.

ANNEX II

Serological methods for detecting plant pathogens

General procedure using antigen-coated plate (ACP)-ELISA

1. Harvest fresh samples of leaf from samples and controls and weigh 0.2 g of each. Grind each sample in 2 ml coating buffer (0.05 M carbonate buffer)+2% w/v polyvinyl pyridine and 0.2% w/v Na_2SO_3. Transfer the sap to a labelled Eppendorf tube. Spin for five minutes at 10 000 rpm.
2. Label the microtitre plate and load the samples, 100 µl per well. Cover the plate with parafilm and incubate at 4ºC overnight. Weigh 4 g of the healthy sample and grind in 10 ml phosphate-buffered saline solution with Tween (PBST), which acts as a blocking solution. It is made by dissolving 80.0 g NaCl, 2.0 g KH_2PO_4, 11.0 g Na_2HPO_4 and 2.0 g KCl in 2000 ml distilled water, adjusting the pH to 7.4 and adding 5.0 ml Tween 20 to make up to 10 l. Weigh the samples and dissolve. Filter through cotton wool and make the filtrate up to 80 ml. Use this to make dilutions of the antiserum. Allow to absorb at 4°C overnight.
3. Make a 0.1% solution of bovine serum albumen in PBST.
4. Rinse the plate in a gentle stream of tap water and wash three times with PBST.
5. Add PBST to all wells of the plate, 150 µl per well. Cover the plate with parafilm and incubate for 30 minutes at room temperature.
6. Completely empty the blocking solution from the plate. Without washing, add the diluted antiserum; use 100 µl per well. Cover the plate with parafilm and incubate three to four hours at room temperature.
7. Make the dilution of the alkaline phosphatase enzyme conjugate in PBST (1/1000 made fresh).
8. Wash the plate three times with PBST, then add the diluted conjugate according to the pattern on the loading diagram, starting with the most diluted. Use 100 µl per well. Cover the plate with parafilm and incubate at 4°C overnight.
9. Wash the plate with PBST three times.
10. Dissolve the substrate tablet (p-NPP, Sigma) in substrate buffer (1mg/ml) (diethanolamine 10%, pH 9.8, stored in a refrigerator).
11. Add the dissolved substrate to all wells, 150 µl per well. Incubate at room temperature for 30 minutes. Evaluate and score the colour intensity of each well with the ELISA reader.

General procedure using tissue-blot immunoassay (TBIA) on nitrocellulose membranes

1. Collect tissues (leaves, petioles, stems, etc.).
2. For thin tissues such as leaves, roll them into a tight core. For batch samples, bind them together with parafilm.
3. Cut a piece of nitrocellulose membrane (NCM), cut the top left corner and mark a grid with in pen.
4. Hold tissues in one hand and cut with a new razor blade in a steady motion with the other hand to obtain a single-cut surface.
5. Press the newly cut surface onto on grid square of the NCM with a firm but gentle force. Document what material has been blotted into which grid on a prepared form. Continue to blot leaves until all grids are full.
6. Wash the NCM three times with PBST at five-minute intervals.
7. Dilute the antiserum in healthy sap in PBST (dilution 1/500–1/2000) and allow to absorb at room temperature for two hours or 4ºC overnight.
8. Block the NCM in 2 µg/ml polyvinyl alcohol in PBST and incubate one minute at room temperature.
9. Wash as in step 6.
10. Add diluted antiserum and incubate for one hour at room temperature.
11. Wash as in step 6.
12. Add anti-rabbit conjugate (dilution 1/1000–1/5000 in conjugate buffer) and incubate for one hour at room temperature.
13. Wash as in step 6.
14. Add substrate solution (NBT/BCIP).
15. To stop the reaction, wash with de-ionized water.

ANNEX III

Glossary of terms

Absolute humidity: The amount of water vapour present in a unit volume of air, usually expressed in kilograms per cubic meter.

Accession: A distinct, uniquely identifiable sample of seeds representing a cultivar, breeding line or a population, which is maintained in storage for conservation and use.

Accession number: A unique identifier that is assigned by the curator when an accession is entered into a collection. This number should never be assigned to another accession.

Achene: A one-seeded dry indehiscent fruit with the seed attached to the pericarp at only one point.

Active collection: A germplasm accession that is used for regeneration, multiplication, distribution, characterization and evaluation. Active collections are maintained in short- to medium-term storage and usually duplicated in a base collection maintained in medium- to long-term storage.

Bar-coding: A computerized coding system that uses a printed pattern or bars on labels to identify germplasm accessions. Bar-codes are read by optically scanning the printed pattern and using a computer program to decode the pattern.

Base collection: A collection of germplasm that is conserved in long-term, secure storage and is not used as a routine distribution source. Seed is usually stored at sub-zero temperatures and low moisture content.

Base unit: The number of seeds needed to ensure the successful implementation of a procedure such as registration or regeneration.

Breeding line: A group of identical pure-breeding diploid or polyploid organisms that are distinguished from other individuals of the same species by a unique phenotype and genotype.

Capsule: A dry dehiscent fruit derived from an ovary with two or more carpels that splits partly open at maturity.

Characterization: The recording of highly heritable characters that can be easily seen and are expressed in all environments.

Collection: A group of germplasm accessions maintained for a specific purpose under defined conditions.

Cultivar: A crop variety produced by scientific breeding or farmer-selection methods.

Database: An organized set of interrelated data assembled for a specific purpose and held in one or more storage media.

Database management system: A piece of software that controls the organization, storage, retrieval, security and integrity of data in a database—it accepts requests from the application and instructs the operating system to transfer the appropriate data. The major vendors are Oracle, IBM, Microsoft and Sybase. MySQL is a very popular open-source product.

Dehiscent fruits: Fruits that open at maturity to shed their seeds (*see follicle, capsule*).

Descriptor: An identifiable and measurable trait, characteristic or attribute observed in an accession that is used to facilitate data classification, storage, retrieval and use.

Descriptor list: A collection of all individual descriptors of a particular crop or species.

Desiccator: A short glass jar fitted with an air-tight cover and containing a desiccating agent such as silica gel or calcium chloride, above which the material to be dried is supported on a perforated platform.

Distribution: The process of supplying samples of germplasm accessions to breeders and other users.

Documentation: The organized collection of records that describe structure, purpose, operation, maintenance, and data requirements.

Donor: An institution or individual responsible for donating germplasm.

Dormancy: The state in which certain live seeds do not germinate, even under normally suitable conditions.

Exploration: The act of searching for genetic diversity in the field.

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 characteristics whose expression is often influenced by environmental factors.

***Ex situ* conservation:** The conservation of biological diversity outside its natural habitat—in the case of plant genetic resources,

this may be in seed genebanks, *in vitro* genebanks or as live collections in field genebanks.

Field collection: A collection of germplasm maintained as living plants—germplasm that would otherwise be difficult to maintain as seed is commonly maintained in field collections.

Follicle: A dry, single-celled, many-seeded fruit consisting of a single carpel, dehiscing by the ventral suture.

Funicle: A stalk by which an ovule or seed attaches itself to the fruit wall.

Genebank: A centre for conserving genetic resources under suitable conditions to prolong their lives.

Genetic diversity: The variety of genetic traits that result in differing characteristics.

Genetic drift: Changes in the genetic composition of a population when the number of individuals is reduced below the frequency of certain alleles within it.

Genetically modified organism (GMO): An organism whose genetic material has been deliberately altered (*see also transgenic plant).*

Genotype: The genetic constitution of an individual plant or organism.

Germplasm: The genetic material that forms the physical basis of heredity and that is transmitted from one generation to the next by germ cells.

Germination: The biological process that leads to the development of a seedling from a seed. Radicle emergence is the first visible sign of germination, but may be followed by no further growth or by abnormal development. According to ISTA rules, only seedlings showing normal morphology are considered to have germinated.

Germination test: A procedure to determine the percentage of seeds that are capable of germinating under a given set of conditions.

Hard seeds: Seeds that fail to imbibe and germinate when placed in a moist medium because they are impermeable to water.

Humidification: The process in which the moisture content of very dry seeds is raised by placing them in a humid environment; humidification helps to prevent damage to seeds from rapid uptake of water.

Imbibition damage: Damage caused by rapid uptake of water in very dry seeds (see also *humidification*).

Indehiscent fruit: Fruit that does not open at maturity (*see also achene*).

***In vitro* collection:** A germplasm collection maintained as plant tissue ranging from protoplast and cell suspensions to callus cultures, meristems and embryos.

Inventory: A list of samples (and their characteristics) that is stored in a genebank.

Isotherm: A graph showing the relationship between seed moisture content and percentage relative humidity (see also *sorption isotherms*).

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.

Landrace: A crop cultivar that has evolved through many years of farmer-directed selection and that is specifically adapted to local conditions; landraces are usually genetically heterogeneous.

Long-term conservation: The storage of germplasm for a long period, such as in base collections and duplicate collections. The period of storage before seeds need to be regenerated varies, but is at least several decades and possibly a century or more. Long-term conservation takes place at sub-zero temperatures.

Mass maturity: The stage in development at which seeds attain maximum dry weight.

Medium-term conservation: The storage of germplasm in the medium-term such as in active and working collections; it is generally assumed that little loss of viability will occur for approximately ten years. Medium-term conservation takes place at temperatures between 0°C and 10°C.

Micropylar region: The point on a seed that was the orifice (pore) of the ovule.

Moisture content (wet-weight basis): The weight of free moisture divided by the weight of water plus dry matter, expressed as a percentage.

Monitoring: The periodic checking of accessions for viability and quantity.

Monitoring interval: The period of storage between monitoring tests.

Most original sample (MOS): A sample of seeds that have undergone the lowest number of regenerations since the material was acquired by the genebank, as recommended for storage as a

base collection. It may be a sub-sample of the original seed lot or a seed sample from the first regeneration cycle if the original seed lot required regeneration before storage.

Multiplication: The representative sample of an accession grown to multiply the quantity of conserved material for distribution.

Normal germination: Germination in which seedlings show all essential root and shoot structures and are capable of developing into mature plants given favourable conditions.

Obsolete variety: A plant variety that is no longer grown commercially.

Out breeding: Controlled or natural matings among unrelated individuals. Out-breeding may also refer to a species that has specific barriers to selfing or exhibits such inbreeding depression that inbred individuals never reach maturity.

Orthodox seeds: Seeds that can be dried to low moisture content and stored at low temperatures without damage to increase seed longevity.

Pathogen: A living micro-organism such as a virus, bacterium or fungus that causes disease in another organism.

Passport data: Basic information about the origin of an accession, such as details recorded at the collecting site, pedigree or other relevant information that assists in the identification of an accession.

Pedigree: The record of the ancestry of a genetic line or variety.

Pest: An organism regarded as injurious or harmful.

Phenotype: The external appearance of a plant that results from the interaction of its genetic composition (genotype) with the environment.

Phytosanitary certificate: A certificate provided by government plant health personnel to verify that seed material is substantially free from pests and diseases.

Pollination: The process in which pollen is transferred from an anther to a receptive stigma by pollinating agents such as wind, insects, birds, bats, or the opening of the flower itself.

Population: A group of individual plants or animals that share a geographic area or region and have common traits.

Propagule: Any structure with the capacity to give rise to a new plant, whether through sexual or asexual (vegetative) reproduction.

This includes seeds, spores, and any part of the vegetative body capable of independent growth if detached from the parent.

Quarantine: The official confinement of introduced germplasm subject to phytosanitary regulations to ensure that it does not carry diseases or pests injurious to the importing country.

Random sample: A sample drawn at random from a larger group.

Recalcitrant seeds: Seeds that loose viability when dried or stored at low temperatures.

Regeneration: Grow-out of a seed accession to obtain a fresh sample with high viability and numerous seeds.

Regeneration standard: The percentage seed viability at or below which the accession must be regenerated to produce fresh seeds.

Relative humidity (RH): A measure of the amount of water present in the air compared to the greatest amount possible for the air to hold at a given temperature, expressed as a percentage. It differs from *absolute humidity,* which is the amount of water vapour present in a unit volume of air, usually expressed in kilograms per cubic meter.

Safety duplication: A duplicate of a base collection stored under similar conditions for long-term conservation, but at a different location to insure against accidental loss of material from the base collection.

Sample: A part of a population used to estimate the characteristics of the whole.

Sequential germination test: A series of discrete seed tests in which the decision to further test seeds or stop the test depends upon the cumulative result.

Silica gel: An inert chemical that absorbs water from its surroundings and will give up this water by evaporation when heated.

Silique: A dry, dehiscent, elongate fruit composed of two carpels separated by a seed-bearing partition.

Solarization: A non-toxic method of killing weeds and insect pests by covering the ground with layers of clear plastic and allowing the sun to create enough heat.

Sorption isotherm: *See isotherm.*

Storage life: The number of years that a seed can be stored before seed death occurs.

Seed viability: The capacity of seeds to germinate under favourable conditions.

Tetrazolium test: A test for viability in which moist seeds are soaked in a solution of triphenyl tetrazolium chloride.

Threshing: The process of beating plants with a machine or by hand to separate the seeds.

Trait: a recognizable quality or attribute resulting from interaction of a gene or a group of genes with the environment.

Transformation: Genetic alteration of a cell resulting from the introduction, uptake and expression of foreign DNA.

Transgene: A gene used in transformation (see transgenic plants).

Transgenic plants: Plants that have been genetically engineered using recombinant DNA techniques for new characteristics. Transgenic plants are produced by adding one or more genes to a plant genome using a process called transformation.

Viability test: A test on a sample of seeds from an accession that is designed to estimate the viability of the entire accession.

Variety: A recognized division of a species, next in rank below subspecies; it is distinguishable by characteristics such as flower colour, leaf colour and size of mature plant. The term is considered to be synonymous with *cultivar*.

Water potential: The chemical potential of water for reaction or movement. Water potential is important for seed drying because it measures the ability of water to move. Water always moves from areas of high water potential to areas of low water potential.

ANNEX IV

Specialized equipment for seed genebanks

(The list is not exhaustive and mention of suppliers does not necessarily constitute endorsement)

No	Item	Specification/Purpose	Supplier
1	Balance, analytical	Weighing up to four decimal places, required for seed moisture content determination using small samples	**Mettler-Toledo (Schweiz) AG** Im Langacher CH-8606 Greifensee Switzerland Phone: (41) 1 944 45 45 Fax: (41) 1 944 45 10 Email: info.ch@mt.com Web: www.mt.com **Ohaus Corporation** P.O. Box 2033 19A Chapin Road Pine Brook, NJ 07058 USA Phone: (1) 973 377 9000 Fax: (1) 973 593 0359 Email: Sales@Ohaus.com Web: www.ohaus.com **Sartorius AG** Weender Landstrasse 94-108 D-37075 Goettingen Germany Phone: (49) 551 308 0 Fax: (49) 551 308 3289 Email: wt.sales@sartoriuscorp.com Web: www.sartorius.com **Cole-Parmer Instruments Co.** 625 East Bunker Court Vernon Hills, IL 60061-1844 USA Phone: (1) 847 549 7600 Fax: (1) 847 549 1700 Email: sales@coleparmer.com Web: www.coleparmer.com **Fisher Scientific** 2000 Park Land Dr. Pittsburg, PA 15275-9943 USA Phone: (1) 973 467-6511 Fax: (1) 800 926 1166 Web: www.fishersci.com **Osaw Industrial Products Pvt. Ltd.** Osaw Complex, Jagadhri Road P.O. Box No. 42, Ambala Cantt. - 133 001 Haryana India Phone: (91) 171-2699347/2699267 Fax: (91) 171-2699222/2699102 Email: eenquiry@indosaw.com **Thomas Scientific** P.O. Box 99 Swedesboro, NJ 08085 USA Phone: (1) 800-524-0018 Fax: (1) 856-467-3087 Email: global@thomassci.com Web: www.thomassci.com

2	Balance, moisture determination	Combining heating with highly accurate weighing technology to deliver a fast and precise method of moisture analysis	**Mettler-Toledo (Schweiz) AG** Im Langacher CH-8606 Greifensee Switzerland Phone: (41) 1 944 45 45 Fax: (41) 1 944 45 10 Email: info.ch@mt.com Web: www.mt.com **Ohaus Corporation** P.O. Box 2033 19A Chapin Road Pine Brook, NJ 07058 USA Phone: (1) 973 377 9000 Fax: (1) 973 593 0359 Email: Sales@Ohaus.com Web: www.ohaus.com **Sartorius AG** Weender Landstrasse 94-108 D-37075 Goettingen Germany Phone: (49) 551 308 0 Fax: (49) 551 308 3289 Email: wt.sales@sartoriuscorp.com Web: www.sartorius.com **Hoffman Manufacturing Co.** 353 29th Ave. S.W. P.O. Box 547 Albany OR 97321 USA Phone: (1) 541 926 2920 Fax: (1) 541 926 3949 Email: info@hoffmanmfg.com Web: www.hoffmanmfg.com **Seedburo Equipment Co.** 1022 W. Jackson Blvd. Chicago, IL 60607 USA Phone: (1) 312-738-3700 Fax: (1) 312-738-5329 Email: sales@seedburo.com Web: www.seedburo.com

3	Cold rooms – medium/long-term	Cold storage modules made of prefabricated polyurethrane insulating panels; panels should be 150 mm thick and constructed for long-term storage (-20°C) or 75 mm thick and for medium-term storage (+5°C); floor insulated and heavy duty, door-hinged type; heated seal for long-term storage, fitted with transparent PVC strip curtain; refrigeration system to provide adjustable controlled environment of -20°C to 10°C; dehumidification system to provide 30-40% RH where required; control panel displaying temperature and RH provided outside	**BMIL International, Inc.** 61 Broadway Suite 1900 New York NY 10006-2701 USA Phone: (1) 212 898 9699 Fax: (1) 212 514 9234 Email: bmil@bmil.com Web: www.bmil.com **Huurre Group Oy.** PO Box 127 FIN-33101 Tampere Finland Phone: (358) 20 5555 11 Fax: (358) 20 5555 360 Email: info@huurre.com Web: www.huurre.com **Foster Refrigerator** Oldmedow Road King's Lynn Norfolk, PE30 4JU United Kingdom Phone: (44) 1553 691122 Fax: (44) 1553 691447 E-mail: sales@foster-uk.com Web: www.Fosterrefrigerator.co.uk **Watford Refrigeration & Air Conditioning Ltd.** Wiggenhall Industrial Estate Watford, WD1 8AW, UK United Kingdom Phone: (44) 1923 227726 Fax: (44) 1923 233525 Email: sales@watref.co.uk Web: www.watref.co.uk
4	Containers a. Aluminium foil bags b. Aluminium cans, Glass bottles	Aluminium foil bags - laminated, made of three layers: polyester on the outside, aluminium in the middle and polyethylene on the inside, and puncture resistant Cans, sealed or with screw caps fitted with rubber gasket; Glass bottles fitted with polypropylene lids	**Barrier Foil Products Co.** Hollands Mill 61 Shaw Heath Stockport, SK3 8BH United Kingdom Phone : (44) 161 4804007 Fax : (44) 161 4747412 Email: BARRIERFOIL@aol.com **Embarcadero Home Cannery** 2026 Livingston Street Oakland, CA 94606 USA Phone: (1) 510 535 2311 Fax: (1) 510 535 2235 Email: contact_ehcan@hotmail.com Web: www.ehcan.com

5	Data loggers	Continuous logging of temperature and RH data in cold rooms and freerers and to record field obseravtions	**OAKTON Instruments** P.O. Box 5136, Vernon Hills, IL 60061, USA Phone: (1) 888 462 5866 Fax: (1) 847 247 2984 Email: info@4oakton.com Web: www.4oakton.com
6	Dehumidifier	Rotary type with high performance activated silica gel or other desiccant wheel	**Bry-Air Inc.** 10793 St. Rt. 37W Sunbury, Ohio 43074 USA Phone: (1) 740 965 2974 Fax: 740 965 5470 Email : bryair1@aol.com Web: www.bry-air.com **Munters Limited,** Blackstone Road Huntingdon Cambridgeshire PE29 6EE United Kingdom Phone: (44) 1480 432243 Fax: (44) 1480 413147 Email: info@munters.co.uk Web: www.munters.com
7	Distillation apparatus	Distilled water for germination testing etc.	**Cole-Parmer Instruments Co.** 625 East Bunker Court Vernon Hills, IL 60061-1844 USA Phone: (1) 847 549 7600 Fax: (1) 847)549 1700 Email: sales@coleparmer.com Web: www.coleparmer.com **Fisher Scientific** 2000 Park Land Dr. Pittsburg, PA 15275-9943 USA Phone: (1) 973 467 6511 Fax: (1) 800 926 1166 Web: www.fishersci.com **Osaw Industrial Products Pvt. Ltd.** Osaw Complex, Jagadhri Road P.O. Box No. 42, Ambala Cantt. - 133 001 Haryana India Phone: (91) 171 2699347/2699267 Fax: (91) 171 2699222/2699102 Email: eenquiry@indosaw.com **Thomas Scientific** P.O. Box 99 Swedesboro, NJ 08085 USA Phone: (1) 800 524 0018 Fax:(1) 856 467 3087 Email: global@thomassci.com Web: www.thomassci.com

8	Drying cabinet/room	Rotary absorption dehumidifiers with secondary refrigeration equipment to provide an environment of 15°-20°C and 15%-20% RH for seed drying	**Bry-Air Inc.** 10793 St. Rt. 37W Sunbury, Ohio 43074 USA Phone: (1) 740 965 2974 Fax: (1) 740 965 5470 Email : bryair1@aol.com Web: www.bry-air.com **Hoffman Manufacturing Co.** 353 29th Ave. S.W. P.O. Box 547 Albany OR 97321 USA Phone: (1) 541 926 2920 Fax: (1) 541 926 3949 Email: info@hoffmanmfg.com Web: www.hoffmanmfg.com **Huurre Group Oy. PO Box 127** FIN-33101 Tampere Finland Phone: (358) 20 5555 11 Fax: (358) 20 5555 360 Email: info@huurre.com Web: www.huurre.com **Munters Limited,** Blackstone Road Huntingdon Cambridgeshire PE29 6EE United Kingdom Phone: (44) 1480 432243 Fax: (44) 1480 413147 Email: info@munters.co.uk Web: www.munters.com **Watford Refrigeration & Air Conditioning Ltd.** Wiggenhall Industrial Estate Watford WD1 8AW UK Phone: (44) 1923 227726 Fax: (44) 1923 233525 Email: sales@watref.co.uk Web: www.watref.co.uk

9	Electronic scale	Weighing up to two decimal places – required at various stages of the seed-handling	**Hoffman Manufacturing Co.** 353 29th Ave. S.W. P.O. Box 547 Albany OR 97321 USA Phone: (1) 541 926 2920 Fax: (1) 541 926 3949 Email: info@hoffmanmfg.com Web: www.hoffmanmfg.com **Seedburo Equipment Co.** 1022 W. Jackson Blvd. Chicago, IL 60607 USA Phone: (1) 312 738 3700 Fax: (1) 312 738 5329 Email: sales@seedburo.com Web: www.seedburo.com **Cole-Parmer Instruments Co.** 625 East Bunker Court Vernon Hills, IL 60061-1844 USA Phone: (1) 847 549 7600 Fax: (1) 847 5491700 Email: sales@coleparmer.com Web: www.coleparmer.com **Fisher Scientific** 2000 Park Land Dr. Pittsburg, PA 15275-9943 USA Phone: (1) 973 467 6511 Fax: (1) 800 926 1166 Web: www.fishersci.com **Osaw Industrial Products Pvt. Ltd.** Osaw Complex, Jagadhri Road P.O. Box No. 42, Ambala Cantt. - 133 001 Haryana India Phone: (91)171 2699347/2699267 Fax: (91)171 2699222/2699102 Email: eenquiry@indosaw.com **Thomas Scientific** P.O. Box 99 Swedesboro, NJ 08085 USA Phone: (1) 800 524 0018 Fax: (1) 856 467 3087 Email: global@thomassci.com Web: www.thomassci.com
10	Freezer (chest type/ vertical)	Standard domestic freezers providing -20°C for long-term seed conservation	Locally available (e.g. Revco, Kelvinator, Westinghouse and others)

11	Germination cabinet (Germinator)	Providing very high RH levels, illuminated, with diurnal-cycling control allowing independent selection of day/night temperatures and light	**Controlled Environments Limited** 590 Berry Street Winnipeg, Manitoba Canada R3H 0R9 Phone: (1) 204 786 6451 Fax: (1) 204 783 7736 Email: sales@conviron.com Web: www.conviron.com **Hoffman Manufacturing Co.** 353, 29th Ave. S.W. P.O. Box 547 Albany, OR 97321 USA Phone: (1) 541 926 2920 Fax: (1) 541 926 3949 Email: info@hoffmanmfg.com Web: www.hoffmanmfg.com **Seedburo Equipment Co.** 1022, W. Jackson Blvd. Chicago, IL 60607 USA Phone.: (1) 312 738 3700 Fax: (1) 312 738 5329 Email: sales@seedburo.com Web: www.seedburo.com **Weiss Gallenkemp Ltd.** Willowbank House, 84 Station Road Marlow, Buckinghamshire SL7 1NX United Kingdom Phone: (44) 1494 43 43 24 Fax: (44) 1494 43 43 Web: www.weisstechnik.co.uk
12	Global Positioning System (GPS)	Portable and handheld to carry on collecting trips	**Garmin International Inc.** 1200 East 151st Street Olathe, KS 66062-3426 (Kansas City metro area) USA Phone: (1) 913 397 8200 Fax: (1) 913 397 8282 Web: www.garmin.com
13	Grinder (coffee mill)	Grinding small quantities of seeds for moisture determination	Locally available (Braun, Moulinex, etc)

14	Incubator	With diurnal-cycling control allowing independent selection of day/night temperatures	**Percival Scientific, Inc** 505 research Drive Perry, Iowa 50220 USA Phone: (1) 515 465 9363 Fax: (1) 515 465 9464 Email: info@percival-scientific.com Web: www.percival-scientific.com **Weiss Gallenkemp Ltd** Willowbank House, 84 Station Road Marlow, Buckinghamshire SL7 1NX United Kingdom Phone: (44) 1494 43 43 24 Fax: (44) 1494 43 43 Web: www.weisstechnik.co.uk **Cole-Parmer Instruments Co.** 625 East Bunker Court Vernon Hills, IL 60061-1844 USA Phone: (1) 847 549 7600 Fax: (1) 847 549 1700 Email: sales@coleparmer.com Web: www.coleparmer.com **Fisher Scientific** 2000 Park Land Dr. Pittsburg, PA 15275-9943 USA Phone : (1) 973 467 6511 Fax: (1) 800 926 1166 Web: www.fishersci.com **Thomas Scientific** P.O. Box 99 Swedesboro, NJ 08085 USA Phone: (1) 800 524 0018 Fax: (1) 856 467 3087 Email: global@thomassci.com Web: www.thomassci.com
15	Magnifier lamp	Seed cleaning	**Hoffman Manufacturing Co.** 353, 29th Ave. S.W. P.O. Box 547 Albany, OR 97321 USA Phone: (1) 541 926 2920 Fax: (1) 541 926 3949 Email: info@hoffmanmfg.com Web: www.hoffmanmfg.com **Seedburo Equipment Co.** 1022, W. Jackson Blvd. Chicago, IL 60607 USA Phone: (1) 312 738 3700 Fax: (1) 312 738 5329 Email: sales@seedburo.com Web: www.seedburo.com

16	Moisture testers a. Quick meter	Quick assessment of moisture content	**Hoffman Manufacturing Co.** 353 29th Ave. S.W. P.O. Box 547 Albany OR 97321 USA Phone: (1) 541 926 2920 Fax: (1) 541 926 3949 Email: info@hoffmanmfg.com Web: www.hoffmanmfg.com **Seedburo Equipment Co.** 1022 W. Jackson Blvd. Chicago, IL 60607 USA Phone: (1) 312 738 3700 Fax: (1) 312 738 5329 Email: sales@seedburo.com Web: www.seedburo.com
	b. Hygropalm	Non-destructive moisture testing	**Rotronic Instruments (UK) Ltd** Unit 1a, Crompton Fields, Crompton Way, Crawley, West Sussex, RH10 9EE United Kingdom Tel: (44) 1293 571000 Fax: (44) 1293 571008 Web: www.rotronic.co.uk
17	Oven (mechanical/ gravity convection)	Temperature range 30°–200°C, with fan convection and temperature controller with adjustable thermostat and timer	**Cole-Parmer Instruments Co.** 625 East Bunker Court Vernon Hills, IL 60061-1844 USA Phone: (1) 847 549 7600 Fax: (1) 847 549 1700 Email: sales@coleparmer.com Web: www.coleparmer.com **Fisher Scientific** 2000 Park Land Drive Pittsburg, PA 15275-9943 USA Phone: (1) 973 467 6511 Fax: (1) 800 926 1166 Email: sales@fishersci.com Web: www.fishersci.com **Osaw Industrial Products Pvt. Ltd.** Osaw Complex, Jagadhri Road P.O. Box No. 42, Ambala Cantt. - 133 001 Haryana India Phone: (91) 171 2699347/2699267 Fax: (91) 171 2699222/2699102 Email: eenquiry@indosaw.com **Thomas Scientific** P.O. Box 99 Swedesboro, NJ 08085 USA Phone: (1) 800 524 0018 Fax: (1) 856 467 3087 Email: global@thomassci.com Web: www.thomassci.com

18	Purity workboard	Seed cleaning	**Hoffman Manufacturing Co.** 353, 29th Ave. S.W. P.O. Box 547 Albany, OR 97321 USA Phone: (1) 541 926 2920 Fax: (1) 541 926 3949 Email: info@hoffmanmfg.com Web: www.hoffmanmfg.com **Seedburo Equipment Co.** 1022 W. Jackson Blvd. Chicago, IL 60607 USA Phone: (1) 312 738 3700 Fax: (1) 312 738 5329 Email: sales@seedburo.com Web: www.seedburo.com **Osaw Industrial Products Pvt. Ltd.** Osaw Complex, Jagadhri Road P.O. Box No. 42 Ambala Cantt. - 133 001 Haryana India Phone: (91) 171 2699347/2699267 Fax: (91) 171 2699222/2699102 Email: eenquiry@indosaw.com
19	Sealing machine (aluminium foil bags/ Cans)	Constant heat machines that use thermostatic controllers to maintain the element bar at a selected temperature to seal laminated aluminium bags and other materials made from layers of plastic film having different properties and melting points	***Aluminium foil bags*** **Audion Elektro BV** P.O. Box 389 1380 AJ WEESP The Netherlands Phone: (31) 294 491717 Fax: (31) 294 491761 Email: holland@audion.nl Web: www.aud.com **Hulme-Martin Tavak** 317 Guildford Road Bisley Woking Surrey GU24 9BB UK Phone: (44) 1483 476767 Fax: (44) 1483 486343 Web: www.hulmemartin.co.uk ***Can sealer:*** **Embarcadero Home Cannery** 2026 Livingston Street Oakland, CA 94606 USA Phone: (1) 510 535 2311 Fax: (1) 510 535 2235 Email: contact_ehcan@hotmail.com Web: www.ehcan.com

20	Seed blower	Seed cleaning – separation of light weight material from seeds	**Hoffman Manufacturing Co.** 353, 29th Ave. S.W. P.O. Box 547 Albany, OR 97321 USA Phone: (1) 541 926 2920 Fax: (1) 541 926 3949 Email: info@hoffmanmfg.com Web: www.hoffmanmfg.com **Seedburo Equipment Co.** 1022 W. Jackson Blvd. Chicago, IL 60607 USA Phone: (1) 312 738 3700 Fax: (1) 312 738 5329 Email: sales@seedburo.com Web: www.seedburo.com **Osaw Industrial Products Pvt. Ltd.** Osaw Complex, Jagadhri Road P.O. Box No. 42 Ambala Cantt. - 133 001 Haryana India Phone: (91) 171 2699347/2699267 Fax: (91) 171 2699222/2699102 Email: eenquiry@indosaw.com
21	Seed counter	Counting a predetermined number of seeds or registering a count on a preweighed or volumetrically measured portion.	**Hoffman Manufacturing Co.** 353, 29th Ave. S.W. P.O. Box 547 Albany OR 97321 USA Phone: (1) 541 926 2920 Fax: (1) 541 926 3949 Email: info@hoffmanmfg.com Web: www.hoffmanmfg.com **Seedburo Equipment Co.** 1022 W. Jackson Blvd. Chicago, IL 60607 USA Phone: (1) 312 738 3700 Fax: (1) 312 738 5329 Email: sales@seedburo.com Web: www.seedburo.com **Osaw Industrial Products Pvt. Ltd.** Osaw Complex, Jagadhri Road, P.O. Box No. 42 Ambala Cantt. - 133 001 Haryana India Phone: (91)171 2699347/2699267 Fax: (91)171 2699222/2699102 Email: eenquiry@indosaw.com
22	Seed-counting boards	For counting and spacing large seeds in planting medium	**Hoffman Manufacturing Co.** 353 29th Ave. S.W., P.O. Box 547 Albany, OR 97321 USA Phone: (1) 541 926 2920 Fax: (1) 541 926 3949 Email: info@hoffmanmfg.com Web: www.hoffmanmfg.com

23	Seed divider	To prepare representative samples from composite samples	**Hoffman Manufacturing Co.** 353, 29th Ave. S.W. P.O. Box 547 Albany OR 97321 USA Phone: (1) 541 926 2920 Fax: (1) 541 926 3949 Email: info@hoffmanmfg.com Web: www.hoffmanmfg.com **Seedburo Equipment Co** 1022 W. Jackson Blvd. Chicago, IL 60607 USA Phone: (1) 312 738 3700 Fax: (1) 312 738 5329 Email: sales@seedburo.com Web: www.seedburo.com **Osaw Industrial Products Pvt. Ltd.** Osaw Complex, Jagadhri Road, P.O. Box No. 42 Ambala Cantt. - 133 001 Haryana India Phone: (91)171 2699347/2699267 Fax: (91)171 2699222/2699102 Email: eenquiry@indosaw.com
24	Shelving (mobile/ static)	Mobile galvanised steel frame with PVC coating similar to library shelves preferable	**Crown Industrial** 213 Michelle Court San Francisco, CA 94080 USA Phone: (1) 650 952 5150 Fax : (1) 650 873 1495 Email: autodor@crown-industrial.com Web: www.mobileshelving.net **Montel** 225, 4th Avenue, C.P 130 Montmagny, Québec G5V 3S5 Canada Fax: (1) 418 248 7266 Phone: (1) 877 935 0236 Email: system@montel.com Web: www.montel.com

25	Sieves, graded	Seed cleaning and separation	**Hoffman Manufacturing Co.** 353, 29th Ave. S.W. P.O. Box 547 Albany OR 97321 USA Phone: (1) 541 926 2920 Fax: (1) 541 926 3949 Email: info@hoffmanmfg.com Web: www.hoffmanmfg.com **Seedburo Equipment Co.** 1022 W. Jackson Blvd. Chicago, IL 60607 USA Phone: (1) 312 738 3700 Fax: (1) 312 738 5329 Email: sales@seedburo.com Web: www.seedburo.com **Osaw Industrial Products Pvt. Ltd.** Osaw Complex, Jagadhri Road, P.O. Box No. 42 Ambala Cantt. - 133 001 Haryana India Phone: (91)171 2699347/2699267 Fax: (91)171 2699222/2699102 Email: eenquiry@indosaw.com
26	Stereo microscope	Seed quality and health assessment	**Cole-Parmer Instruments Co.** 625 East Bunker Court Vernon Hills, IL 60061-1844 USA Phone: (1) 847 549 7600 Fax: (1) 847 549 1700 Email: sales@coleparmer.com Web: www.coleparmer.com **Fisher Scientific** 2000 Park Land Dr. Pittsburg, PA 15275-9943 USA Phone: (1) 973 467 6511 Fax: (1) 800 926 1166 Web: www.fishersci.com **Osaw Industrial Products Pvt. Ltd.** Osaw Complex, Jagadhri Road, P.O. Box No. 42 Ambala Cantt. - 133 001 Haryana India Phone: (91) 171 2699347/2699267 Fax: (91) 171 2699222/2699102 Email: eenquiry@indosaw.com **Thomas Scientific** P.O. Box 99 Swedesboro, NJ 08085 USA Phone: (1) 800 524 0018 Fax: (1) 856 467 3087 Email: global@thomassci.com Web: www.thomassci.com

27	Storage trays (metal/ plastic)	Should withstand below-freezing temperatures	Local suppliers
28	Supplies, General a. Germination paper b. Labware (petrishes, forceps, desiccator, other glassware etc.)		**Hoffman Manufacturing Co.** 353, 29th Ave. S.W. P.O. Box 547 Albany OR 97321 USA Phone: (1) 541 926 2920 Fax: (1) 541 926 3949 Email: info@hoffmanmfg.com Web: www.hoffmanmfg.com **Seedburo Equipment Co.** 1022 W. Jackson Blvd. Chicago, IL 60607 USA Phone: (1) 312 738 3700 Fax: (1) 312 738 5329 Email: sales@seedburo.com Web: www.seedburo.com **Whatman plc.** 27 Great West Road Brentford Middlesex TW8 9BW UK Main Tel: (44) 208 326 1740 Main Fax: (44) 208 326 1741 Email: information@whatman.com **Cole-Parmer Instruments Co.** 625 East Bunker Court Vernon Hills, IL 60061-1844 USA Phone: (1) 847 549 7600 Fax: (1) 847 549 1700 Email: sales@coleparmer.com Web: www.coleparmer.com **Fisher Scientific** 2000 Park Land Drive Pittsburg, PA 15275-9943 USA Phone: (1) 973 467 6511 Fax: (1) 800 926 1166 Web: www.fishersci.com **Osaw Industrial Products Pvt. Ltd.** Osaw Complex, Jagadhri Road, P.O. Box No. 42, Ambala Cantt. - 133 001 Haryana India Phone: (91) 171 2699347/2699267 Fax: (91) 171 2699222/2699102 Email: eenquiry@indosaw.com **Thomas Scientific** P.O. Box 99 Swedesboro, NJ 08085 USA Phone: (1) 800 524 0018 Fax: (1) 856 467 3087 Email: global@thomassci.com Web: www.thomassci.com

	b. Field (seed envelops, pollination bags, labels, etc.) c. Chemicals (Tetrazolium chloride, Agar, desiccants, etc.)		**A.P. Burt & Sons** Severn Paper Mill Portishead Bristol BS20 7DJ UK Phone: (44) 1275 842454 Fax: (44) 1275 84 96 13 **PBS International** Salter Road Scarborough YO11 3UZ UK Tel : (44) 1723 584091 Fax : (44) 1723 581664 Emal : pbs@duraweld.co.uk Web : www.pbs.co.uk **M/S Ajay Kumar & Co.** C-149 Moti nagar New Delhi 100 015 India Tel : (91) 11 5100776 Fax : (91) 11 5441950 **Sigma-Aldrich** Customer Service PO Box 14508 St. Louis, MO 63178 USA (See Web site for other countries) Phone: (1) 800 325 3010 Fax: (1) 800 325 5052 Web: www.sigmaaldrich.com **Merck Chemicals Ltd.** Boulevard Industrial Park Padge Road, Beeston Nottingham NG9 2JR UK Phone: (44) 115 9430840 Fax: (44) 115 9574237 Email: information@merckchem.co.uk

29	Thermohygrometer, hygrothermograph	To monitor temperature and RH in clod rooms	**Cole-Parmer Instruments Co.** 625 East Bunker Court Vernon Hills, IL 60061-1844 USA Phone: (1) 847 549 7600 Fax: (1) 847 5491700 Email: sales@coleparmer.com Web: www.coleparmer.com **Fisher Scientific** 2000 Park Land Dr. Pittsburg, PA 15275-9943 USA Phone : (1) 973 467 6511 Fax: (1) 800 926 1166 Web: www.fishersci.com **Thomas Scientific** P.O. Box 99 Swedesboro, NJ 08085 USA Phone: (1) 800 524 0018 Fax: (1) 856 467 3087 Email: global@thomassci.com Web: www.thomassci.com
30	Thresher, mechanical	Designed for threshing single plants and heads of small cereal grain	**Hoffman Manufacturing Co.** 353, 29th Ave. S.W. P.O. Box 547 Albany OR 97321 USA Phone: (1) 541 926 2920 Fax: (1) 541 926 3949 Email: info@hoffmanmfg.com Web: www.hoffmanmfg.com **Seedburo Equipment Co.** 1022 W. Jackson Blvd. Chicago, IL 60607 USA Phone: (1) 312 738 3700 Fax: (1) 312 738 5329 Email: sales@seedburo.com Web: www.seedburo.com

ANNEX V

List of acronyms

ACIAR	Australian Centre for International Agricultural Research
ACP	antigen-coated plate
AOSA	Association of Seed Analysts
CBD	Convention on Biological Diversity
CGIAR	Consultative Group on International Agricultural Research
CGN	Centre for Genetic Resources, The Netherlands
CITES	Convention on International Trade in Endangered Species of Wild Flora and Fauna
CPB	Cartagena Protocol on Biosafety
CTA	Technical Centre for Agriculture and Rural Cooperation ACP-EU
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
eRH	equilibrium relative humidity
FAO	Food and Agriculture Organization of the United Nations
GAA	germplasm acquisition agreement
GMO	genetically modified organism
GPS	global positioning system
GRPC	Genetic Resources Policy Committee
IBPGR	International Board for Plant Genetic Resources (now Bioversity International)
ILRI	International Livestock Research Institute
IP	intellectual property
IPGRI	International Plant Genetic Resources Institute (now Bioversity International)
IPPC	International Plant Protection Convention
ISTA	International Seed-Testing Association
MCPD	multi-crop passport descriptor
MOS	most original sample
MTA	material transfer agreement
NASH	nucleic acid spot hybridization
NCM	nitrocellulose membrane
PBST	phosphate-buffered saline solution with Tween
PCR	polymerase chain reaction
PGRFA	plant genetic resources for food and agriculture
RH	relative humidity
RNA	ribonucleic acid
SMC	seed moisture content
SMTA	Standard Material Transfer Agreement for species included in Annex I of the International Treaty on PGRFA
SPGRC	SADC Plant Genetic Resources Centre
TBIA	tissue-blot immunoassay
UPOV	International Union for the Protection of New Plant Varieties
WA	agar solution
WorldVeg	AVRDC—The World Vegetable Center

IPGRI and INIBAP
operate under the name
Bioversity International

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