Gene Banking for Ex Situ Conservation of Plant Genetic Resources

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Gene Banking for *Ex Situ* Conservation of Plant Genetic Resources

23

P.E. Rajasekharan

Abstract

Seed storage is one of the most widespread and valuable *ex situ* approaches to conservation. Extensive expertise has been developed in this field by agencies and institutions involved with plant genetic resources over the past 30 years. Seed banking has considerable advantages over other methods of *ex situ* conservation such as ease of storage, economy of space, relatively low labour demands and, consequently, the capacity to maintain large samples at an economically viable cost. Depending on the species, seeds are dried to suitably low moisture content according to an appropriate protocol. Typically this will be less than 5 %. The seeds then are stored at −18 °C or below. Because seed DNA degrades with time, the seeds need to be periodically replanted and fresh seeds collected for another round of long-term storage. There are about six million accessions, or samples of a particular population, stored as seeds in about 1,300 gene banks throughout the world as of 2006. The procedure for seed storage along with the latest development on gene banking is discussed in this chapter.

Keywords

Gene bank, accessions seeds community seed bank, permafrost

23.1 Introduction

The former Soviet Union gained an early lead in collecting and conserving plant genetic resources as a result of the work of Vavilov, who was responsible for the extensive and valuable collections assembled at the All-Union Institute of Plant Industry at St. Petersburg (formerly Leningrad). The Vavilov All-Union Institute of Plant Industry (VIR), as it was to be known later, became the central nationwide institution responsible for collecting and conserving global plant diversity and studying it for the purposes of plant breeding and crop improvement in the Soviet Union (Vavilov 1997) In the United States, collection and evaluation of germplasm has been

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performed since the 1900s, but the first repository of crop seeds was not established until 1947 when the Regional Plant Introduction Station in Ames, IA, began operation. In 1958, the first national facility for storage of germplasm at low temperatures in cold rooms was constructed at Fort Collins, CO. These facilities were recently upgraded (Qualset and Shands 2005). An IBPGR survey in 1975 revealed that there were only eight long-term genetic resources' conservation centres globally (almost all of them in the industrialised countries). But only 7 years later, the total had jumped to 33, and today there are over 1,000 major germplasm collections in gene banks all over the world (Qualset and Shands 2005). Crop genetic resources grown from true seed are stored in three main types of gene banks. In long-term gene banks, whose aim is to store material for 50–100 years, samples are kept at −10 °C to −20 °C in airtight containers. Usually before such storage, the seed samples are dried to a moisture content of 5–7 %. In medium-term facilities, as most working collections are stored, temperatures of 0–5 °C are maintained and seeds may last up to 10–15 years. The short-term collections or breeder's collections are usually kept in paper envelopes or cloth bags or tin cans at 5–15 °C without any seed drying. Samples are constantly withdrawn for evaluation. Much of the germplasm of major crops and their wild and weedy relatives already resides in gene banks. The effect of the vast increase in accessions since the 1970s on germplasm utilised for crop production has thus far been modest (Damania 2008). More than a decade ago, a US survey found around three-quarters of soy and wheat breeders and around three-fifths of cotton and sorghum breeders, but only 45 % of corn breeders used gene banks more than 'rarely'.

Since the 1970s, work on the conservation of crop genetic resources has increasingly become a large-scale independent activity detached from crop improvement efforts. A substantial germplasm collecting effort was launched in the 1970s in response to concerns about genetic erosion and crop vulnerability. Over 1,000 gene banks have been established, holding about six million accessions (FAO 1998). The germplasm holdings in major international agricultural research centres are provided in Table 23.1. Seed banks of a global network of international agricultural research institutions, coordinated by the Consultative Group on International Agricultural Research (CGIAR), Washington, are focused on crops and have extensive seed collections for such crops as rice, maize, wheat, barley, millets, pulses, oil seeds, tuber crops, banana, tropical forage and fruits. The collections in these seed banks are well documented, and the institutions are networked among themselves and with several other institutions. The Millennium Seed Bank Project (MSBP) at the Royal Botanic Gardens, Kew, England, is one of the largest conservation projects. MSBP's 47 partner organisations in 17 countries intend to store 25 % of the world's plant species by 2020. The Seed Information Database (SID) at Kew is an ongoing compilation of seed characteristics and traits worldwide, targeted at >24,000 species.

What makes seed banks such an effective *ex situ* conservation technique is that the methodology can be applied to a wide range of species in a universal and straightforward way and that large amounts of intraspecific diversity can be conserved and for long periods of time without intervention. Additionally, germinating seeds to obtain fully grown plants is relatively simple compared with obtaining plants from *in vitro-stored* material. Plants recovered from banked seeds can also be compared with natural populations from which the material was harvested

Table 23.1 PGR holdings at IARCs (Source: SINGER)

Name of IARC	Number of accessions
AVRDC	52,845
Bioversity International	1,208
CIAT	72,246
CIMMYT	120,527
CIP	15,092
ICARDA	140,189
ICRAF	1,785
ICRISAT	114,865
IITA	27,596
ILRI	20,177
IRRI	108,272
WARDA	21,752

years before and which may have been subjected subsequently to environmental change (e.g. as a result of global warming).

23.2 Current Status of Seed Banks for Food and Agriculture

Just under half of the six million accessions are held in 12 national collections. To some degree, this is a function of the early establishment of their genetic resource collections. The collections include those in Russia and the United States (noted earlier), Japan (established in 1966), Germany (1970), Canada (1970) and Brazil (1974). Of the 1,308 national or regional collections currently noted by FAO (1996), only 397 within 75 countries are held in long- or medium-term seed banks. Medium-term storage might be assumed to be in the order of 10 or more years.

23.3 Scientific Principles Underlying Seed Banking

Viable seeds of many species when maintained in a dry and cold state are capable of being germinated many years later. This capability means that the long-term *ex situ* conservation of many higher plants is a realistic possibility.

23.3.1 Seed Storage Conditions

The science of seed storage is not a new one and dates back, at least, to China in the sixth century. Advances in the quantification of seed longevity under different storage conditions were made in the second half of the twentieth century through the work of Harrington in the United States and Ellis and Roberts in the United Kingdom (see review in Hong, Jenkins et al. 1998). Critical factors that determine seed longevity are the seed's moisture content, temperature, and gaseous environment; its initial viability; and its genetic background. With respect to the latter, differences between species would appear to be much greater than those within. Genetics particularly influences the relationship between seed longevity and seed moisture content. Most species produce seeds that can be dried to low moisture contents (e.g. where less than 5 % of the seeds' fresh weight is water) without loss of viability. The seeds of such species are termed 'orthodox'.

23.3.2 Genetic Considerations

The genetics of seed bank storage is an important issue. A criticism occasionally levelled at seed banks is that there is selection in storage. Selection can occur during collection through a biassed sample (e.g. for early- or late-flowering genotypes). It can also occur when samples are grown out under conditions that differ dramatically from those where the seeds were harvested. There is, however, little evidence to show that, compared with, say, room conditions, the seed bank environment does other than slow down the (normally distributed) times for individuals to die. In other words, there is no greater risk of selection out of any individual by the conditions applied than under natural conditions.

23.4 Other Forms of Gene Banks

23.4.1 Conservation of Pollen

Conservation of nuclear genetic diversity (NGD) using pollen is desirable in horticultural species for a variety of reasons. Cryopreserved pollen can be a major access point for pre-breeding germplasm lines, hybrid seed production and biotechnological and other basic studies. In the case of tree species, germplasm can be easily received and exchanged through pollen, eliminating a long juvenile phase. The objective of a useful pollen cryostorage protocol is to collect mature pollen from plant and treat it so as to retain its normal function, ultimately assessed by its ability to germinate in vivo and effect normal fertilisation (Hanna and Towill 1995). Alexander and Ganeshan (1993) reviewed the work on pollen storage in fruit crops. Hoekstra (1995) has

assessed the merits and demerits of pollen as genetic resource. Ganeshan and Rajasekharan (1995) reviewed work on ornamental crop pollen storage. Grout and Roberts (1995) detailed the methodology for pollen cryopreservation. Recently, Barnabas and Kovacs (1997) and Berthoud (1997) stressed the importance and need for pollen conservation. Response to cryopreservation of pollen of 45 species belonging to 15 families is presented in Table 23.1. In some of the recently cryostored pollen, only feasibility tests were carried out. In most species, protocols are optimised for establishing pollen cryobanks.

Besides the already existing role of pollen banks in breeding, there are many promising applications which have come to focus with the recent advances in allied bioscientific areas. Some of the practical utilities are discussed below:

23.4.2 Advantages to the Use of Pollen

- Gene-banked pollen can be made available to breeders upon request. For tree species, this obviates the need for growing the male parents in the breeding orchards. It allows for wide hybridisation across seasonal and geographical limitations and reduces the coordination required to synchronise flowering and pollen availability for use in crosses. With adequate pollen available, one can also load additional pollen onto stigmas to increase pollination and yield.
- Pollen is available for research programmes. As single cells, pollen provides a simple model system for research on conservation. Storage of pollen within gene banks also ensures its availability year-round for basic biology and allergy research programmes (Shivanna 2003).
- Pollen captures diversity within small sample sizes, and documentation is available for long-term survival of pollen from many diverse species. Pollen also serves as a source of genetic diversity in collections where it is hard to maintain diversity with seeds (species of low fecundity, large seeds, or seeds that require an investment of labour to store).
- Pollen can also be shipped internationally, often without threat of disease transfer (Hoekstra 1995) (http://cropgenebank.sgrp.cgiar.org/).

Detailed protocols for pollen cryopreservation have been given by Ganeshan et al. (2008) and Rajasekharan et al. (2013).

23.4.3 Conservation of DNA

Advances in genomics have provided technologies for high-throughput analysis of plant genomes with potential for use in gene discovery in germplasm collections. Biotechnology has made possible DNA conservation of plant species, in the form of extracted DNA or as genomic DNA libraries. However, this technique needs advanced technological inputs to match with the importance and requirement for the species in question, to justify its need as a practical conservation strategy. For any given plant species, DNA conservation could be advantageous for:

1. The study of molecular phylogenetics and systematics of extant and extinct taxa
2. Production of previously characterised secondary compounds in recombinant DNA-mediated transgenic cell cultures
3. Production of transgenic plant using genes from gene families
4. *In vitro* expression and study of enzyme structure and function, synthesising genomic probes for research laboratories

The establishment of DNA banks facilitates this screening by making DNA from large numbers of plant accessions widely available. DNA banks require the development of appropriate policies for access and benefit sharing. Tools for automating sample and data handling are essential. Standard molecular methods for fingerprinting DNA accessions for international comparisons need to be determined. New screening technologies are required to take advantage of the emerging availability of large DNA collections.

The Australian Plant DNA Bank aims to collect DNA from all Australian plant species and to sample the diversity within each species. DNA from all individuals of the species is being stored for rare species. Domesticated or economically important species from all countries are also being collected and stored. The international networking of DNA banks will be a key step in linking genomics tools to global plant diversity (Rice et al. 2006).

23.4.3.1 The Use of DNA Banks

Molecular techniques are becoming increasingly important in the study and management of genetic resources. DNA has been routinely extracted and stored from the nuclei, mitochondria and chloroplasts of many plant species, together with derivatives such as RNA, cDNA and genes. Technologies are available to allow all these to be stored quickly and at low cost in DNA banks as an insurance policy against loss of crop diversity. DNA storage has so far been undertaken with objectives other than conservation in mind, usually to allow genetic material to be made readily available for molecular applications, for distribution or for training (http://cropgenebank.sgrp.cgiar.org/).

23.4.3.2 Advantages

DNA banking is an efficient, simple and long-term method to conserve the genetic information.

23.4.3.3 Disadvantages

There are problems with subsequent gene isolation, cloning and transfer of DNA back to a plant, and it currently does not allow the regeneration of the same genotype as the original sample.

23.4.3.4 Storage

There is little information on the long-term stability of extracted DNA during frozen storage, but most repositories consider several years to decades as realistic. Information on the stability of purified DNA dissolved in buffer suggests that the overall fragment size decreases with storage time and that the usefulness of the specimen for PCR-based assays may be 1–2 years when stored at 4 °C, 4–7 years when stored at −18 °C and greater than 4 years when stored at −80 °C (Madisen et al. 1987; Visvikis et al. 1998). The choice of temperature usually depends on the moisture level within the sample.

23.5 Community Seed Banks (CSBs)

Community seed banks fulfil diverse purposes of sustainable agriculture for small and marginal farmers. These seed banks serve as focal point in maintaining indigenous genetic diversity on farms involving farmer community. CSBs serve local farmers to form an informal seed distribution system prevailing in villages since ancient time at no or very low cost. Community participation in maintaining local genetic diversity provides pride to farmers and sense of belonging for local landraces. This system is run, maintained and promoted by farmers to facilitate good quality seeds and input (Malik et al. 2013). There are no set guidelines available to establish and manage community seed banks as they form an important part of informal seed distribution system in villages since ancient time. The farming community as per their convenience has developed this system and the same is being continued by the farmers.

23.6 Soil Seed Bank

The soil seed bank is the natural storage of seeds, often dormant, within the soil of most ecosystems. The study of soil seed banks started in 1859 when Charles Darwin observed the emergence of seedlings using soil samples from the bottom of a lake. The first scientific paper on the subject was published in 1882 and reported on the occurrence of seeds at different soil depths. Weed seed banks have been studied intensely in agricultural science because of their important economic impacts; other fields interested in soil seed banks include forest regeneration and restoration ecology.

23.7 Seed Bank Management

23.7.1 Procedure

The basic elements of the seed banking procedure (more or less in order) are as follows (Linington et al. 2001):

- Collection planning and permission seeking
- Seed (and pressed specimen) collecting and field data recording
- Shipment of seeds
- Creation of a data record about the accession
- Seed cleaning (sometimes preceded by initial drying and sometimes accompanied by X-ray analysis and quantity determination)
- Main drying
- Seed moisture determination
- Initial germination test (sometimes left until after banking)
- Packaging and banking and security duplication
- Characterisation (including verification of identity in the case of wild species) and evaluation (where appropriate)
- Distribution of stocks to users (through time)
- Germination retests (through time)
- Regeneration/multiplication (as required)

23.7.2 Seed Collection

Set against the background of the CBD and the International Undertaking, collecting should only be carried out with the permission of the national and local authorities. In India as per the Biodiversity Act, one has to take permission from local bodies for collection. Foreign collectors should work collaboratively with local scientists, and clear agreements on benefit sharing should be in place. One immediately tangible benefit is the sharing of collections and the information relating to them. Other international regulations need to be adhered to. These include the Convention on International Trade in Endangered Species (CITES) and national quarantine laws. Seed collecting methodology and genetic resource exploration have been thoroughly covered by Guarino et al. (1995). In most instances, random and even sampling of wild plant or crop populations is recommended, including careful note taking of the sample method (in often less than perfect conditions). Objective data recording is essential as is accurate recording of location. This latter aspect is now facilitated by the use of Global Positioning System receivers that help fix latitude, longitude and even altitude using satellites.

23.7.3 Seed Cleaning

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

Seed cleaning is necessary to:

- Reduce bulk during transportation.
- Improve purity of the sample.
- Optimise storage space and reduce costs.
- Prevent seed from going mouldy and help reduce ‘damping off’ or fungal contamination after germination.
- Allow precise regulation of seed moisture content during storage. Seeds should be cleaned immediately after harvest or soon after they arrive at the gene bank. Cleaning methods vary according to the type of seed.

Cleaning should not cause damage to samples or lead to waste. It can be done manually or by machines, but gene banks 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.

23.7.4 Seed Bank Storage Standards

Seeds can be classified into the following types according to seed storage behaviour:

1. *Orthodox* – seeds that can withstand low seed moisture content and can be stored at low temperature without losing viability
2. *Recalcitrant* – seeds that lose viability if moisture content is less than 12–13 %
3. *Intermediate* – seeds that exhibit storage characteristics between orthodox and recalcitrant seeds

For orthodox seeds, the storage potential is influenced by inherent and external factors; for example, some legumes that are hard-seeded are long-lived, while seeds with high oil content are short-lived. There is also observed variation at the eco-geographic races (e.g. *indica* vs. *japonica* rice) and cultivar levels. Seeds harvested at their physiological maturity generally store better. The environment (temperature, moisture, nutrition and light) under which the crop is planted can also affect storability. The longevity of seeds depends on the initial seed quality, moisture content and temperature during storage. In general, low moisture content and low temperature reduce the loss of seed viability. Harrington's rule of thumb can be applied as rough estimate of length of storage in reference to temperature and seed mc. The rule states that beginning at 14 % mc, for every 1 % decrease, the lifespan of the seeds doubles, and beginning at 50 °C, for every 5 °C decrease in storage temperature, the lifespan of the seeds doubles.

No two banks are the same. Traditionally, seed banks have been classified into the following categories:

- *Base* collections that are for the long-term storage of seed lots and from which seeds are not normally sent to users (though this is not always the case).
- *Active* collections from which seeds are made available to users. Often such stores are maintained under less optimal storage conditions compared to those holding base collections though this need not be the case. FAO/IPGRI (1994) recommends that storage lives of 10–20 years might be appropriate.

23.7.5 Seed Drying

Seed drying is the reduction of seed moisture content to the recommended levels (which should not be lower than the critical seed moisture content) for storage using techniques which are not detrimental to seed viability. Dry seeds retain viability for longer periods during storage. Seeds are hygroscopic and absorb or desorb moisture depending on the relative humidity of the surrounding air or 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 becomes drier (desorption). If the water vapour pressure of the seed is lower than the surrounding air, the seed will gain moisture (absorption). Absorption or desorption occurs until the water vapour pressure in the seed and the surrounding air is balanced. The water content of seeds at equilibrium with the RH of surrounding air is referred to as equilibrium moisture content. Understanding the relation between equilibrium seed moisture content and relative humidity is important for the gene bank technician to decide on the drying regime for seeds. Several methods are available for drying seeds. Methods that minimise loss of viability during drying should be used. The most common and safe methods used for drying are dehumidified drying and silica gel drying. Drying rate depends on seed size, shape, structure, composition, initial seed moisture content, amount of seeds and layers, air movement, temperature and relative humidity.

23.7.6 Packaging

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

Seeds are packaged to:

- Prevent absorption of water from the atmosphere after drying.
- Keep each accession separate and avoid the mixing of accessions.
- Prevent contamination of the seeds from insects and diseases.

The best time to package seeds is immediately after the moisture content has been determined and found to be within the required limits for safe storage. Dry seeds will reabsorb moisture from ambient air. Therefore, seeds should be packaged into containers and hermetically sealed without delay, soon after removal from the drying room or cabinet. Different types of containers are available for packaging. The choice depends on storage conditions and species. The most important thing is that the packing material should be completely impermeable to water and suitable for long-term use. Some frequently used containers in gene banks are glass bottles, aluminium cans, aluminium foil packets and plastic bottles. These different types of containers all have advantages and disadvantages. Glass bottles are good but fragile and can easily break. Aluminium cans are difficult to reseal once they are opened. Aluminium foil can be resealed and occupy less space in storage room. However, seeds with sharp projection can pierce the packets and moisture can leak inside. Plastic bottles are moisture resistant but not moisture proof. They should be used with caution if relative humidity of the storage room is not controlled. Packaging is best carried out in an air-conditioned room where the relative humidity is controlled. It is important to ensure that seeds taken from the drying room are exposed to the ambient air for the shortest possible time so that they do not reabsorb water.

23.7.7 Storage Temperature

Many long-term seed banks store seed under deep-freeze conditions using either purpose-built prefabricated cold rooms or domestic deep freezers. To reduce staff time at subzero temperatures, a few seed bank cold rooms, such as one at the National Institute of Agrobiological Resources (NIAR), Japan, have mechanised banking/retrieval systems. The uses of such systems have implications to energy consumption by the bank. Use of permafrost has been considered for long-term duplicate storage of seed in places such as Svalbard. Stores are usually unable to match the lowering of temperatures possible in conventional base storage conditions. In 1997, the Japanese-based Biological and Environmental Specimen Time (BEST) Capsule 2001 Project discussed the possibilities of long-term storage of flagship samples under Antarctic ice at 58 °C (which incidentally is not sufficiently low for animal tissue preservation) or even on the dark side of the moon at 230 °C.

23.7.8 Monitoring Seed Lot Viability

Perhaps one of the most important parameters of seed bank effectiveness is the result of germination monitoring. Germination is the preferred test for seed lot viability. Providing such information to those using the seed is helpful. Additionally, other viability tests such as vital staining using tetrazolium solution have a greater element of subjectivity about them. This staining test is, however, sometimes used to help distinguish between dead and dormant seeds among those that did not germinate under a given test regime. Two problems relate to the germination monitoring of seed bank accessions: First, because seeds are tested soon after arrival at the bank and then at regular intervals (often every 5–10 years) during their storage life, the tests need to be repeatable and operator independent. Second, in order to recover as many genotypes as possible represented within a seed lot, it is necessary to break seed dormancy. This can be a particular problem in the seed of wild species and where the seed is freshly harvested. Key techniques include scarification of hard seed coats to facilitate water or oxygen permeation, imbibed chilling at 5–10 °C and later incubation at diurnal alternating temperatures with fluorescent light (i.e. rich in red light) provided only in the higher temperature phase. Tests have to be seed lot specific as most seed dormancy is not strongly genetically inherited and the form it takes depends on the conditions under which the seed matured. Once determined, the same treatments can be used during the monitoring of that seed lot through time.

23.7.9 Duplication

One of the main advantages of seed banks is that they centralise collections of genetic material making them more easily accessed and studied. Indeed, some seed banks might be seen as some of the world's greatest plant diversity hot spots with more individuals and, in some banks, more species per square metre than anywhere else on the planet. This centralisation poses a risk to all but the most carefully located and constructed facilities. Potential catastrophic loss, which of course threatens plants, conserved both *ex situ* and in situ, means that duplication of collections and their associated data is an important element of seed bank safety. The FAO report (1996) indicates that the level of security duplication of plant genetic resources for food and agriculture still needs to be improved and is at best uncertain.

23.7.10 Characterisation and Evaluation

Characterisation can vary from accurate naming of the species or subspecies represented by the collection through to more detailed recording of characters governed by genes that are little modified by environmental factors (major genes). Such information, published in the form of descriptor lists, is of great value to plant breeders wishing to narrow their choice of material from, often, vast collections. Similarly, the concept of core collections has been established to facilitate use by breeders. A core collection genetically represents a limited set of accessions of a crop gene pool with the minimum repetition. Increasingly, characterisation is taking the form of more detailed molecular techniques such as screening by amplified fragment length polymorphism. By contrast to characterisation, evaluation records data on traits such as yield that are strongly influenced by the environment in which the plants are grown. Such data are thus site and year specific and are perhaps of less use to breeders.

23.7.11 Distribution to Users

A very important element of seed bank work is to make the seed available wherever possible. In evidence of the scale of such dispatch, Kerry Ten Kates and Laird (1999) quote an annual distribution of nearly 120,000 samples by the US National Plant Germplasm System, of which 65 % are sent abroad, many requested by reference to the Germplasm Resources Information Network (GRIN) available on the Internet. Furthermore, the usage rate of crop banks by plant breeders is probably less than the rate of request from banks holding broader plant diversity collections where uses include a wide array of pure and applied research in addition to field trials. During 1994–1996, there was a 50 % request rate for seeds offered through an extensive list offered by the Kew Seed Bank.

23.7.12 Regeneration

Seed bank accessions are grown out for the purposes of regeneration of seed stock (either when seed numbers are low or when viability has reduced), for characterisation and for evaluation. Many banks have a regeneration standard below which the germination of a seed lot should not fall. This is usually set at 85 %. This high value limits the risk of accumulated genetic damage that is associated with seed ageing. Even though falling levels of seed germination are correlated with falling levels of field establishment, many banks have adopted lower standards. This may in part be due to the backlog of regeneration work that in some national facilities highlighted by FAO (1996) is nearly 100 % of the collection. By collecting high-quality seed lots in good quantity, other banks have reduced the necessity for regeneration that can be time- and labour-consuming and that can have adverse effects on the genetics of the collection. Samples regenerated under conditions different from where they originated can experience selection. If too small a sample is regenerated, genetic drift may occur in which

rarer alleles are lost through chance. Under some circumstances, recollection, if possible, may be the more desirable option.

23.8 Seed Bank Design

Having considered the aspects of seed bank management, a brief consideration of seed bank design is appropriate (also see Cromarty et al. 1985). The location of the bank is important from political, practical and security aspects. Potential risks have to be considered be they earthquake, flooding or radiation fallout. Some facilities are placed underground such as the seed bank at Krasnodar in Russia and the Millennium Seed Bank in the United Kingdom. Others such as the NSSL are located on the first floor to limit possible impact from structures above resultant from seismic activity. The size of most banks should be dictated by peak annual intake (seed drying and cleaning facilities), projected capacity before a rebuild which is practical (seed storage) and annual collection maintenance (germination, field and greenhouse facilities). Cold storage facilities vary from a few domestic deep freezers up to large rooms such as one of 140 m^2 (with capacity for 150,000 samples) at NIAR in Japan.

23.9 Gene Bank Standards

The *Genebank Standards for Plant Genetic Resources for Food and Agriculture* is intended as a guideline for gene banks conserving plant collections (seeds, live plants and explants). They were developed based on a series of consultations with a large number of experts in seed conservation, cryopreservation, *in vitro* conservation and field gene banks worldwide. The standards are voluntary and nonbinding and have not been developed through standard-setting procedure. They should be viewed more as targets for developing efficient, effective and rational *ex situ* conservation in gene banks that provides optimal maintenance of seed viability and genetic integrity, thereby ensuring access to and use of high-quality seeds of conserved plant genetic resources (FAO 2014).

The standards, as described in this document, define the level of performance of a routine gene bank operation below which there is a high risk of losing genetic integrity. Each section is divided into:

A. Standards
B. Context
C. Technical aspects
D. Contingencies

Specific standards have been developed for eight key areas and include the following sections:

- Acquisition and initial handling
- Testing for nonorthodox behaviour and assessment of water content, vigour and viability
- Hydrated storage for recalcitrant seeds
- *In vitro* culture and slow growth storage
- Cryopreservation
- Documentation
- Distribution and exchange
- Security and safety duplication

An operation manual is also available for gene bank (Rao et al. 2006) (Tables 23.2, 23.3, 23.4 and 23.5).

Promoting genetic resources' utilisation, precise evaluation and documentation of plant genetic resources is a prerequisite for their utilisation. The following areas of research need to be paid more attention for promoting effective utilisation of PGR gene resources:

Table 23.2 PGR holdings in the National Gene Bank, India

Crop group	No. of accessions
Cereals	145,765
Minor millets	53,466
Pseudo-cereals	6,619
Grain legumes	56,870
Oilseeds	54,994
Fibre crops	11,483
Vegetables	24,112
Fruits	382
Medicinal and aromatic crops	6,304
Spices and condiments	2,708
Agroforestry	2,433
Safety duplicates	10,235
Total	*375,371*

Source: NBPGR website

Table 23.3 Details of various *ex situ* conservation sites for PGRFA in India

Type of conservation and nodal ministry	Number of department facilities
Seed gene bank (long-term collections, −18 °C), Ministry of Agriculture	ICAR 1
Seed gene bank (medium-term collections, 4 °C), Ministry of Agriculture	ICAR 28
Seed gene bank (short-term collections at around 10 °C), Ministry of Agriculture	ICAR 13
Botanical gardens, Ministry of Environment	BSI 150
In vitro conservation (4–25 °C), Ministry of Agriculture	ICAR 5
Field gene bank, Ministry of Agriculture	ICAR, SAU 25
Cryopreservation (using liquid nitrogen), Ministry of Agriculture	ICAR 2

Table 23.4 Number of samples of PGR distributed over the last 10 years

Year	No. of samples
1996	20,775
1997	27,022
1998	23,313
1999	11,064
2000	9,714
2001	10,771
2002	12,274
2003	15,487
2004	15,543
2005	9,366
2006	9,537
Total	*164,866*

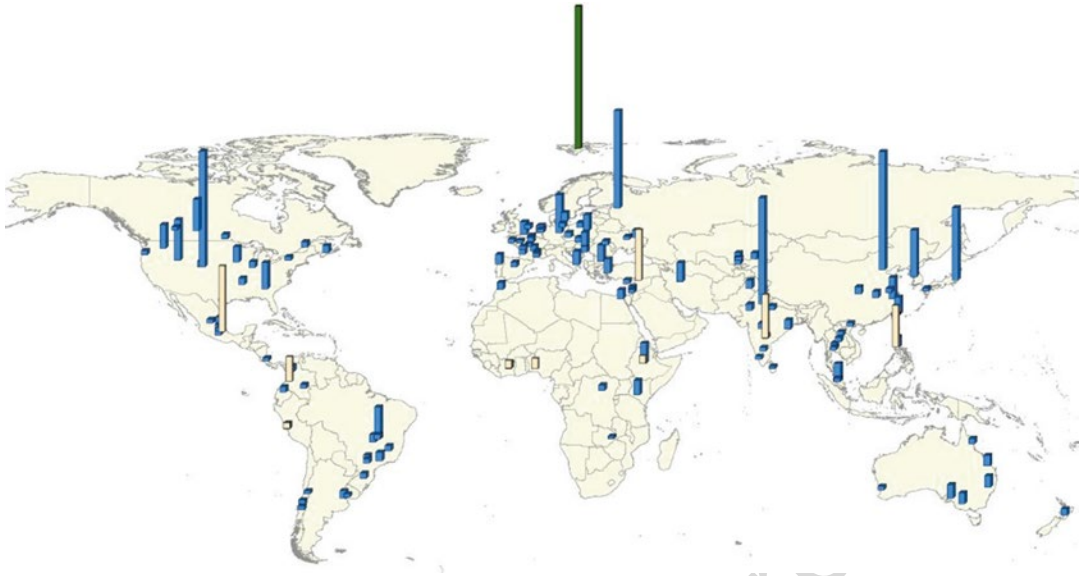
- Development of core collections
- Focused Identification of Germplasm Strategy (FIGS)
- Pre-breeding
- Gene prospecting and allele mining for a trait of interest from genetic resources (Tables 23.6 and 23.7)

On 18 April 2013, the Commission on Genetic Resources for Food and Agriculture (CGRFA) of the United Nations Food and Agriculture Organization (FAO) endorsed and adopted the revision of the *Genebank Standards*, last published in 1994. The revised *Genebank Standards* take into account the changes in *ex situ* conservation conditions, diversity in storage requirements and purpose and period of germplasm conservation, ranging from temperate to tropical provenances. Field gene banking is the most commonly used method for nonorthodox seed-producing plants, for plants that produce very few.

Seeds are vegetatively propagated and/or have a long life cycle, and their standards have been defined accordingly. The standards for *in vitro* culture and cryopreservation are broad and generic in nature, due to the marked variation among nonorthodox seeds and vegetatively propagated plants (Tyagi and Agrawal 2013).

23.10 Economic Considerations

The annual cost (in year 2000 US$) of conserving and distributing the genetic material presently held in all 11 CGIAR gene banks is estimated to be 5.7 million US$ (m US$), which could be maintained for all future generations by setting aside a fund of 149 m US$ (invested at a real rate of interest of 4 % per annum) (Virchow 2003). This would be sufficient to underwrite the costs for the CGIAR's current conservation activities in perpetuity (estimated to be 61 m US$), as well as the cost of maintaining the distribution activities (88 m US$) that provide germplasm to breeders, scientists, farmers and others worldwide (Koo et al. 2003).



Gene banks of the world

Table 23.5 Total accessions conserved *ex situ* and the number of safety duplicates for various categories of crop species (1996–2005)

Sl. no.	Status	No. of crop spp.	No. of accessions	Safety duplicates as active collections	Safety duplicates (%)
1.	Traditional cultivar/landrace	280	121,274	84,931	70
2.	Wild	314	15,881	4,745	30
3.	Weedy	70	267	11	4
4.	Breeders line	37	14,661	2,272	15
5.	Mutant/genetic stock	26	7,898	4,880	62
6.	Advanced/improved cultivar	59	9,080	4,867	54
7.	Others	73	27,662	2,378	9

Table 23.6 Genetic diversity collection and utilisation

Commodity	Landraces	% in collections	Wild species	In situ collections	*Ex situ* collections (acc.)	Utilisation distribution
Rice	140,000	90	20	Few	420,000	High
Maize	65,000	90	–	Few	277,000	High
Sorghum	45,000	80	20	Few	169,000	Low
Millets	30,000	80	–	None	90,000	Low
Soybean	30,000	60	–	None	174,000	Low-medium
Chickpea	22,230[a]	90	19	None	33,782[a]	High
Pigeon pea	8,220[a]	80	57	Few	13,628[a]	Medium-high
Groundnut	6,374[a]	90	45	None	15,419	Medium-high
Potato	30,000	95	65	Few	31,000	High

Modified after Evenson et al. (1998)

[a]ICRISAT and ICARDA holdings

t7.1 **Table 23.7** Status of base collections in the National
t7.2 Gene Bank of India [NBPGR, New Delhi (−18 °C)] (as of
t7.3 28 February 2014; Source: NBPGR website)

t7.4 t7.5 Crop/crop group	Number of accessions conserved
t7.6 Paddy	97,314
t7.7 Wheat	40,340
t7.8 Maize	9,639
t7.9 Others	12,276
t7.10 *Cereals*	*159,569*
t7.11 Sorghum	20,542
t7.12 Pearl millet	8,930
t7.13 Minor millet	22,614
t7.14 Others	5,437
t7.15 *Millets and forages*	*57,523*
t7.16 Amaranth	5,579
t7.17 Buckwheat	886
t7.18 Others	389
t7.19 *Pseudo-cereals*	*6,854*
t7.20 Chickpea	17,373
t7.21 Pigeon pea	11,432
t7.22 Mung bean	3,705
t7.23 Others	26,246
t7.24 *Grain legumes*	*58,756*
t7.25 Groundnut	14,937
t7.26 *Brassica*	10,759
t7.27 Safflower	8,081
t7.28 Others	24,700
t7.29 *Oilseeds*	*58,477*
t7.30 Cotton	7,329
t7.31 Jute	2,915
t7.32 Others	2,214
t7.33 *Fibre crops*	*12,458*
t7.34 Brinjal (eggplant)	4,119
t7.35 Chilli	2,012
t7.36 Others	19,199
t7.37 *Vegetables*	*25,330*
t7.38 Custard apple	59
t7.39 Papaya	23
t7.40 Others	448
t7.41 *Fruits*	*530*
t7.42 Opium poppy	350
t7.43 *Ocimum*	473
t7.44 Tobacco	1,490
t7.45 Others	4,559
t7.46 t7.47 *Medicinal and aromatic plants and narcotics*	*6,872*
t7.48 Coriander	911
t7.49 *Sowa* (dill)	91
t7.50 Others	2,845
Crop/crop group	Number of accessions conserved
Spices and condiments	*3,847*
Pongamia (oil tree)	395
Others	2,048
Agroforestry	*2,443*
Lentil	7,712
Pigeon pea	2,523
Duplicate safety samples	*10,235*
Total	*402,894*[a]

Table 23.7 (continued)

No. of crop species conserved: 1,586 t7.51

[a]The figure includes 4,305 released varieties and 2,300 genetic stocks; regenerated accession not included t7.52 t7.53

23.11 Svalbard

Svalbard Global Seed Vault: On 26 February 2008, the Svalbard Global Seed Vault (SGSV) opened near Longyearbyen (Norway), 600 miles from the North Pole. SGSV is designed to hold 4.5 billion batches of seeds of the world's main crops. The SGSV is a glazed cave-like structure, drilled 500 ft below permafrost, in the middle of a frozen Arctic mountain topped with snow, with the goal to store and protect samples from every seed collection in the world, which will stay frozen. An automated digital monitoring system controls temperature and humidity and provides high security. The SGSV is an insurance against natural disasters, such as earthquakes and tsunamis, or deliberate attacks like bomb blasts or human errors such as nuclear disasters or failure of refrigeration that may erase the seeds of any important species in the other seed banks or in the wild, in the other countries. Such seed can be re-established using seeds from SGSV. NBPGR facilitated safe transfer of about 40,000 accessions of ICRISAT mandate crops to SSGV during the past 2 years.

The Svalbard Global Seed Vault was established with the 'objective to provide a safety net for the international conservation system of plant genetic resources, and to contribute to the securing of the maximum amount of plant genetic diversity of importance to humanity for the long term in accordance with the latest scientific knowledge and most appropriate techniques'.

Ensuring that the genetic diversity of the world's food crops is preserved for future generations is an important contribution towards the reduction of hunger and poverty in developing countries. This is where the greatest plant diversity originates and where the need for food security and the further development of agriculture is most urgent. The Svalbard Global Seed Vault, which is established in the permafrost in the mountains of Svalbard, is designed to store duplicates of seeds from seed collections around the globe. Many of these collections are in developing countries. If seeds are lost, e.g. as a result of natural disasters, war or simply a lack of resources, the seed collections may be re-established using seeds from Svalbard. The loss of biological diversity is currently one of the greatest challenges facing the environment and sustainable development. The diversity of food crops is under constant pressure. The consequence could be an irreversible loss of the opportunity to grow crops adapted to climate change and new plant diseases, and the seeds of an expanding population of more than 200,000 crop varieties from Asia, Africa, Latin America and the Middle East—drawn from vast seed collections maintained by the Consultative Group on International Agricultural Research (CGIAR)—will be shipped to a remote island near the Arctic Circle, where they will be stored in the Svalbard Global Seed Vault (SGSV), a facility capable of preserving their vitality for thousands of years.

23.12 Conservation Benefits

The value of genetically coded information can never be determined a priori but rather only from a posterior observation as a result of their success on the market. To assign monetary value to the benefits generated by PGR conserved in ex situ collections is exceedingly difficult because they have multiple dimensions. The use of germplasm in plant breeding leads to changes in crop output and breeder's requests represent the demand for germplasm in terms of crop in production and trade. Little information is available on the germplasm movement. Only some gene banks and national programmes have drawn up some statistics. Over the past 3 years, for instance, the CGIAR centres have distributed an annual average of over 50,000 accessions to national programmes all over the world (SGRP 1996). Similarly between 1992 and 1994, the Unites States distributed over 100,000 samples each year (FAO 1996). In developing countries, 34 % of all accessions are stored in public gene banks, 49 % in developed countries, 1 % private companies and 0.2 % local conservators (Iwanaga 1993). In India, Ethiopia and China, the expected value for PGRFA conservation is assessed as being very high (Virchow 1999). The benefits of conservation must be analysed more in depth to guide the conservation investment on national and international levels. It is necessary to define the conservation objectives for adjusting these to some cost-efficiency criteria. The conservation investments must follow the criteria of cost efficiency. Objectives must be quantified. In the accessibility of germplasm for present and future use, the link between conservation and utilisation and distribution and characterisation has to be strengthened (http://cropgenebank.sgrp.cgiar.org/).

References

Alexander MP, Ganeshan S (1993) Pollen storage. In: Chadha KL, Pareek OP (eds). Advances in horticulture, vol I. Fruit crops part-I. Malhotra Publishing House, New Delhi 110 064. pp 481–496

Barnabas B, Kovacs G (1997) Storage of pollen. In: Shivanna KR, Sawney VK (eds) Pollen biotechnology for crop production and improvement. Cambridge Univ Press, Cambridge, pp 293–314

Berthoud J (1997) Strategies for conservation of genetic resources in relation with their utilization. Euphytica 96:1–12

Crop gene bank knowledge base. http://cropgenebank.sgrp.cgiar.org/

Cromarty AS, Ellis RH, Roberts EH (1985) The design of seed storage facilities for genetic conservation. IBPGR, Rome

Damania AB (2008) History, achievements, and current status of genetic resources conservation. Prev Publ Agron J 100:9–21

Evenson RE, Gollin P, Santaniello V (eds) (1998) Agricultural values of plant genetic resources. CABI Publishing, Wallingford

FAO/IPGRI (1994) Gene bank standards. Food and Agriculture Organization of the United Nations/International Plant Genetic Resources Institute, Rome

[AU1]

FAO (1996) Report on the State of the World's Plant Genetic Resources for Food and Agriculture prepared for the International Technical Conference on Plant Genetic Resources Leipzig, Germany, Plant Production and Protection Division FAO Via delle Terme di Caracalla 00100 Rome. 17–23 June

FAO (1998) The state of the world's plant genetic resources for food and agriculture. FAO, Rome

FAO (2004) International plant genetic resources treaty. FAO, Rome

FAO (2014) Genebank standards for plant genetic resources for food and agriculture. Rev. ed. Rome

FAO/IPGRI (1) Genebank standards. FAO and IPGRI, Rome. Available at: ftp://ftp.fao.org/docrep/fao/meeting/015/aj680e.pdf)

Ganeshan S, Rajasekharan PE (1995) Genetic conservation through pollen storage in ornamental crops. In: Chadha KL, Bhattacharjee SK (eds) Advances in horticulture vol-12-Part-I. Ornamental crops. Malhotra Publishing House, New Delhi, pp 87–108

Ganeshan SPE, Rajasekharan PE, Shashikumar S, Decruze W (2008) Cryopreservation of pollen plant cryopreservation: a practical guide. Springer, Netherlands, pp 443–464

Grout BWW, Roberts AV (1995) Storage of free pollen, pollen embryos and the zygotic embryos of seed by cryopreservation and freeze drying. In: Grout B (ed) Genetic preservation of plant cells *In Vitro*. Springer, Berlin, pp 63–74

Guarino L, Ramanatha Rao V, Reid R (eds) (1995) Collecting plant genetic diversity: technical guidelines. CAB International on behalf of IPGRI in association with FAO, IUCN and UNEP, Wallingford, p 748

Hanna WW, Towill LE (1995) Long-term pollen storage. In: Janick J (ed) Plant breeding reviews. Wiley, Chester, pp 179–207

Hong TD, Jenkins NE, Ellis RH, Moore D (1998) Limits to the negative logarithmic relationship between moisture-content and longevity in conidia of Metarhizium flavoviride. Ann Bot 81(5):625–630

Hoekstra FA (1995) Collecting pollen for genetic resource conservation. In: Guarino L, Ramanatha Rao V, Reid R (eds) Collecting plant genetic diversity. Technical guidelines. CAB International, Wallingford, pp 527–550

Iwanaga M (1993) Enhancing the links between germplasm conservation and use in a changing world. Int Crop Sci 1:407–413

Koo B, Pardey PG, Wright BD (2003) The economic costs of conserving genetic resources at the CGIAR centres. Agric Econ 29(2003):287–297

Linington Simon H, Hugh W, Pritchard (2001) Encyclopedia of biodiversity, vol 3. Academic, Cambridge, MA

Madisen L, Hoar DI, Holroyd CD, Crisp M, Hodes ME, Reynolds JF (1987) DNA banking: the effects of storage of blood and isolated DNA on the integrity of DNA. Am J Med Genet 27:379–390

Malik SK, Singh PB, Singh A, Verma A, Ameta N, Bisht IS (2013) Community seed banks: operation and scientific management. National Bureau of Plant Genetic Resources, New Delhi, p 64

Rice N, Cordeiro G, Shepherd M, Bundock P, Bradbury L, Pacey-Miller T, Furtado A, Henry R (2006) DNA banks and their role in facilitating the application of genomics to plant germplasm. Plant Genet Resour 4(1):64–70

Qualset CO, Shands HL (2005) Safeguarding the future of US Agriculture: the need to conserve threatened collections of crop diversity worldwide. University of California, DANR/GRCP, Davis

Rajasekharan PE, Ravish BS, Vasantha Kumar T, Ganeshan S (2013) Pollen cryobanking for tropical plant species. In: Conservation of tropical plant species. Springer Verlag, Berlin/Heildelberg/New York, pp 65–75 6

Rao NK, Hanson J, Dulloo ME, Ghosh K, Nowell D, Larinde M (2006) Manual of seed handling in genebanks, vol 8, Handbooks for Genebanks. Bioversity International, Rome

SGRP (1996) Report of the Internationally Commissioned External Review of the CGIAR Genebank operations. IPGRI (International Plant Genetic Resources Institute), Rome

Shivanna KR (2003) Pollen biology and biotechnology. Science Publishers, Enfield, pp 204–210

Ten kate K, Laird SA (1999) The commercial use of biodiversity; access to genetic resources and benefit sharing. Earthscan, London, p 398

Tyagi RK, Agrawal A (2013) Genebank standards – revised guidelines adopted by FAO. Curr Sci 104:1600–1601

Vavilov NI (1997) Origin and geography of cultivated plants. Cambridge University Press, Cambridge, UK

Virchow D (1999) Conservation of genetic resources cost and implications for a sustainable use of plant genetic resources for food and agriculture. Springer, Berlin/New York

Virchow Detlef (ed) (2003) Efficient conservation of crop genetic diversity theoretical approaches and empirical studies. Springer-Verlag, Berlin/Heidelberg/New York

Visvikis S, Schlenck A, Maurice M (1998) DNA extraction and stability for epidemiological studies. Clin Chem Lab Med 36:551–555

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