

Seed Collection and Storage

A strategy for *ex-situ* conservation of flora in Western Australia

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INTRODUCTION

Ex-situ conservation refers to the maintenance of samples of organisms away from their natural habitat and may take the form of seed, pollen, vegetative propagules, tissue or cell culture, living plants or DNA. These living tissue are collectively termed germplasm. Genebanks are the long term storage facilities that conserve this genetic material. The use of seed-based genebanks as an *ex-situ* technique for the conservation of biodiversity is generally well established in most countries, especially for crop species and recently becoming more common for wild native species.

Threatening processes such as loss of habitat, weed invasion, salinity and disease (in particular dieback disease - *Phytophthora* spp.) are major factors in the local extinction of native plant species in Western Australia. A changing climate will interact with many of these stressors to further threaten native flora. Seedbanking is used as an interim solution to prevent this loss of genetic diversity and as a last resort in preventing the extinction of the species. Loss of populations and substantial reduction in population size may not necessarily lead to immediate extinction but usually results in loss of genetic diversity. Species survival and evolutionary processes rely on the maintenance of genetic variation. In the wild this variation enables plants to adapt to changing environmental and ecological conditions, as well as providing resistance to pests and disease.

Seedbanking is complimentary to *in-situ* conservation and can be considered a means to an end rather than an end in itself. It allows access to biodiversity material for research both on- and off-season thereby removing pressure off *in-situ* populations and allowing monitoring of the species. The highly compact nature of seeds make them ideal for storage, and seed storage is more economical than maintaining collections of living plants in botanic gardens. Material is available for the re-establishment or re-stocking of populations or communities of plants back into the wild should difficulties occur in managing *in-situ* populations due to environmental disaster. *Ex-situ* conservation is a cheap insurance policy and, in some cases, represents the only option available if the remaining natural populations are to be conserved in the face of destruction of their habitat. Seed banks are a sensible compromise between technical difficulty, cost and ability to conserve over the long term substantial genetic variation of a range of plant species.

Long term commitment is required to undertake *ex situ* conservation and presupposes an acceptance of a responsibility for the conservation of genetic diversity.

Management of sizeable germplasm collections requires a systems approach, well-defined objectives, adequate facilities, competent staff and sustained administrative and financial support.

Seed banking works because:

- Plants occur in populations and can produce seed in quantity.
- Seeds are naturally dispersed and removal of a proportion of the seed from a wild population is not harmful.
- Seeds are small in relation to the parent plant: the genetic potential of many individuals or an entire population can be contained in a small volume.
- Wide species applicability.
- Seeds of most species are desiccant tolerant and can be dried and frozen.
- Potentially large storage life.
- The genetic potential of plant populations can be held in seedbanks for many years (decades/ centuries) and can be released for propagation in the future.
- The technology is relatively straight forward.
- Cost effective means to conserve genetic diversity.

Despite the benefits of seedbanking, there are a number of controversial issues surrounding this conservation strategy. One argument against *ex situ* collections is that they fail to evolve in response to changing selection pressures that may act on wild populations. The collection and storage of genetic material as a conservation strategy can also lead to people thinking that it is a substitute for conservation of wild populations, providing a clear mandate for destruction of those species/populations that have already been seen to be conserved in a genebank.

The Threatened Flora Seed Centre (TFSC) has been operating as a long term germplasm storage facility for threatened Western Australian plant taxa for over 15 years, having been established in late 1992 by the then Department of Conservation and Land Management. The objective of the Threatened Flora Seed Centre is to ensure the maintenance of genetically representative seed collections of Western Australian threatened flora under long term storage conditions as an interim solution to the prevention of genetic degradation or local extinction of critical populations. Seed collection aims to systematically capture 90-95% of the common alleles from each threatened taxon on a representative range-wide basis. This broad sample of genetic variation is essential if the stored material is to be effectively used in the long-term re-establishment of the species in the wild following removal of the threats.

SEED COLLECTION

Correct sampling is one of the fundamental pillars of good conservation policy. Seed collections for conservation purposes should be taken from wild sources. Collecting from cultivated plants grown in a garden may mean that undesirable selection occurs for those characteristics that directly or indirectly relate to cultivation. Genetic diversity may be lost. Genetic variation is the raw material that increases the chance of a target species surviving thorough changed circumstances in off-site collections, and that makes the material suitable for storage, commercial use or translocation.

Ideally before sampling or collecting from natural populations a survey should be made to map the variation within the species across its geographical and ecological range. Such an eco-geographical survey involves considerable desk and field research but allows for decisions to be made as to the areas to be sampled, probable number of samples needed and sampling strategy. In most cases this will not be feasible or practical and sampling will have to be done on an *ad hoc* basis. The population should be assessed for size, density and extent if this information is not already known from the available literature. Seed should be collected from across these populations, or provenances.

The provenance concept is based on evolutionary genetic principles and recognises that local populations have evolved and adapted to local conditions. The general consensus being that provenance material from local populations is likely to be the most suitable for site rehabilitation and land management, especially in terms of long term survival and ecological processes. At the intra-specific level, genetic variation between populations may reflect critical reproductive and physiological differences that are not evident at the taxonomic level. The exact definition of a population in the field is far from simple, although for rare plants it is likely that they occur in small and disjunct groups or as isolated occurrences. It is important to note that within a population there may be ecotypic variation that requires sampling.

Timing of Collections

Seed collections should be timed to coincide with maturation of the fruit, ideally at the point of natural dispersal. At this point seeds of most species will have attained desiccation tolerance enabling them to be dried to low moisture contents for long term storage. Flowering, fruit ripening and seed shed can vary from species to species and often along an altitudinal and longitudinal gradient. Seed development may take from weeks to over a year dependent on the species. The timing of seed collection is crucial for species whose mature fruits are held for only short periods prior to dispersal (eg. *Acacia* species). Prevailing environmental factors (temperature and moisture) have a major influence on time to maturity. For example, hot, windy conditions may speed the onset of maturity in fruit and seed release; cold, wet conditions may lengthen fruit and seed development. Serotinous plants (e.g. *Banksia*, *Hakea*, many *Dryandra*, *Allocasuarina* and many small-seeded myrtaceous species like *Callistemon*, *Calothamnus* and *Melaleuca*) retain seed on the plant within woody fruits for several years and the timing of collection is less important.

Data on reproductive biology (eg. flowering and fruiting time, fruit characteristics and seed dispersal mechanisms) for target species and climatic conditions for target locations are valuable for formulating collecting procedures and need to be taken into account when deciding on the timing of collections. Fruiting and seed dispersal information is often unavailable and reconnaissance of populations during flowering is recommended. The emergence of some species is dependent on disturbance events such as fire or may be reliant on factors such as adequate rainfall at certain times of the year. The collection strategy will need to take such factors into consideration.

Collecting Strategy

The aim of a collecting strategy should be to conserve genetic variation at the species, population, individual and allelic levels. Genetic diversity provides the basis for

adaptation of an organism to its existing environment and its potential for adaptation to future environmental changes. Loss of genetic variability can diminish the adaptive ability of a species. Structuring collecting within and between populations should aim to capture a significant proportion of the variation possessed by a species. Sampled seeds should contain similar levels of genetic diversity and viability as the plants from which they were collected from. A genetically representative sample can be achieved by directing collection activities over a number of known populations of a species and by collecting from a wide range of individual plants covering ecotypic and morphological variation within each population. Molecular data, if available, can also be used as a guide to ensure that collections are representative of genetic variation within species.

Seed should always be collected from wild sources, unless the species is extinct in the wild or if collection will further threaten populations. Collecting from cultivated plants grown in a garden is not advisable as they may represent limited genetic diversity. Plant material of unknown origin and limited genetic diversity are inadequate for representing the diversity of a population or species. Collecting for horticultural or display purposes may warrant selecting material for a desirable trait, such as colour, shape or form, rather than for a representation of the genetic diversity of the species.

No collecting should be carried out without an appropriate licence and permissions from landholders. It is illegal to collect any plant material (seeds, herbarium specimens, cuttings etc) in Western Australia without a licence unless on your own private property (although it is not permissible to collect Declared Rare Flora on your own property without a licence). Departmental wildlife officers, under the authority of the Wildlife Conservation Act 1950, can take you to court for any offence against native flora.

An effective seed collection strategy should address the following issues:

- what species should be collected?
- how many populations of that species should be collected?
- how many individuals within each population should be collected?
- how much seed from each individual should be collected?

- **What species**

The degree of threat, the geographic range of the species, the number of individuals and populations, and the conservation status of the species may all influence the species targeted for collection.

- **Number of Populations per Taxa**

For conservation taxa, all populations should be sampled if the number of populations is less than five, and at least five if the number of populations exceeds five. As many different and diverse sites as possible should be sampled within a species range if time and resources permit.

- **Number of Source Plants per Population**

Seeds should be collected from at least 50 individual source plants within a population. If the population contains fewer than 50 individuals, it is recommended

that seed should be harvested from all seeding individuals, taking no more than 10-20% of the available seed crop. There is a need to be aware that plants may appear separate but are in fact clonal and may be a single genotype. Where the species is widely distributed and many populations can be sampled, a maximum of 50 samples per population, reflecting ecological and geographical variation, may be sufficient. The objective is to collect a genetically representative sample of the population without damaging its prospects for survival in the wild.

- **Number of Seed per Source Plant**

The ideal number of seeds required to adequately represent and conserve a genepool under long term storage conditions, and provide sufficient resources for the implementation of a solid translocation program ranges from 10,000-20,000 viable seeds per population. This should also allow sufficient seeds for research, education and distribution if necessary.

- **Multi-year Sampling**

Quantities of seed sufficient for long term storage may be accessed by implementing a repetitive (albeit benign) seasonal sampling regime until an adequate sample is obtained. This may require multi-season or multi-year sampling.

If collection of seed from some species proves very difficult (due to small population sizes, low and sporadic seed production, high seed predation levels and disturbances, such as disease, fire or drought, which cause temporal genetic fluctuations in the genepool), seed traps are a viable option. These can be simply calico or paper bags or nylon stockings placed over developing fruits, or else more sophisticated seed traps made from steel posts and flyscreen wire suspended around and beneath the plants. The latter is a very efficient and effective method for the collection of seeds from species that fruit over long periods of time.

The key elements of recommended collecting strategy

- ◆ Carefully assess the population to determine that it is a natural population and not a planted one on a road verge or a previous revegetation effort.
- ◆ Make sure of species identification and be aware that plants can hybridise or look similar.
- ◆ Look for plants that are healthy not diseased
- ◆ Examine seed for quality and quantity (insect damage, empty seed, maturity).
- ◆ Where population size permits, randomly and evenly sample at least 50 individuals. Preferably sample more than 50 individuals, as this may increase genetic diversity and boost seed quantity. Record the number of individuals sampled.
- ◆ Aim to collect from genetically unrelated plants (ie. not adjacent plants) if the population is widely distributed. Closely spaced individuals may be related. Consider the impact of pollinators and dispersal mechanisms on your target species.
- ◆ Stratified random sampling should be used when the population exhibits local variation (ecologically, geographically and/or morphologically). Keeping ecotypes separate, goes further in ensuring that alleles of particular ecological significance are collected.
- ◆ For threatened species or populations with 20 or less individuals, samples from individual plants should be kept separate, so maternal lines can be monitored in future reintroductions.
- ◆ Don't over collect (no more than 20% of seed available per individual or population). This ensures that the population sampled is not endangered by the planned seed collecting. The only exception to this is if a population is going to be destroyed

- ◆ Collect ripe or mature fruits/ seed into either breathable cloth or paper bags that are well secured.
- ◆ Collect entire seed heads of awned or similar species into paper bags.
- ◆ Collect fleshy fruits directly into plastic bags, and allow them to aerate so they don't decompose and become mould infested.
- ◆ Collect herbarium specimen (s) to verify taxonomic identification. If plant numbers are too low photos may suffice.
- ◆ Collect in dry weather if possible to reduce fungal contamination and spread of soil-borne disease such as *Phytophthora cinnamomi*.
- ◆ Collect only seed or fruit. Avoid collecting other plant material as the risks of herbivory and fungal growth are increased.
- ◆ Always label containers and do not damage seed.
- ◆ Always obtain necessary permits and permissions.
- ◆ Always record what has been done and include information on plant morphology, habitat and population characteristics.
- ◆ Pass information on to relevant agencies.

During collecting, precautions should be taken to ensure good population sampling, that documentation is thorough, and that seeds are stored temporarily under conditions which will maintain maximum viability until incorporation into long term storage facilities. Seed should be collected into permeable bags made of cloth or paper. Bags should be well secured at the top with adequate labelling inside and out. If the plant has shed its seeds and collection from the ground is necessary this fact should be recorded so that it is known that these seeds are of indeterminate age and origin. It is necessary to be aware that seeds collected from the ground may have been predated.

The removal of insect pests and fleshy material that may encourage fungal or bacterial growth and rotting should be attempted as soon as possible after collection. If collecting when material is wet, seeds may need to be dried in the field. Air-drying or use of silica gel may be warranted. Seeds should be kept cool and dry at all times. Steps should be taken to ensure that seeds reach a seed bank as soon as possible after collection.

SEED GERMINATION/VIABILITY TESTING

Once the collection or accession has been cleaned and fumigated it should undergo germination/viability determination. Representative sub-samples need to be obtained from each accession sufficient for these tests. In many cases the optimum pre-treatment for germination of plant species will be unknown due to poorly understood seed biology and testing for dormancy breaking might be required. A range of dormancy-breaking techniques such as the use of the growth hormone Gibberellic Acid, smoke, heat or cold stratification and/or heat treatment (wet or dry) may need to be trialed to aid germination.

SEED STORAGE

Seed longevity varies greatly among species, also among collections within a species due to different genotypes and provenance. Provenance affects longevity due to

cumulative effects of the environment during seed maturation, harvesting, drying and pre-storage conditions. On average, the seed longevity of wild species is less than that of crop species when stored under identical conditions. This may have to do with a lack of information on seed quality and maturation, seed dormancy etc of wild species. In crop plants, selection has taken place for uniformity of seed maturation, and for lack of dormancy. Proposed longevity is based on seed ageing experiments, but these data are extrapolated as seed ageing experiments are long term.

Information on seed storage behaviour may be unavailable for many species, but it is possible to use a protocol to determine how seed will survive and for how long under various storage conditions. In general, seeds keep better at relatively low temperatures than at high temperatures, though seeds vary widely in their sensitivity to temperature. Fluctuations are more unfavourable to preserving quality and longevity than even temperatures. A few species can survive for long periods of time under practically all conditions, some under only certain conditions, others are extremely hard to preserve for more than a few years, while others lose viability within weeks.

Drying

Seed should be dried before storage for several reasons:

- Storage is prolonged.
- Seed can tolerate extreme temperatures.
- Germination is prevented.
- Attack by insects, mites and fungi are reduced.

The drying process can be considered as a form of short term storage during which seed moisture content declines. The treatment the seed receives during moisture content decline can cause deterioration of seed. Delays in drying seed or drying seed slowly at high relative humidity and temperatures above 25°C can reduce the viability of seed (especially oily ones). Drying must be benign to the seed, simple to operate and applicable to many seed types and species. The provision of a drying room maintained at 15°C and 15% relative humidity with good air circulation using an air dehumidifier and refrigeration is the simplest solution for large quantities of seed (but costly). Drying with silica gel in a closed desiccator at 15-20°C is acceptable and much cheaper. The minimum equipment needed to dry seeds for storage is a shelf in arid regions or a desiccator elsewhere. Seed can be dried in the shade with good ventilation during dry seasons at ambient temperature and humidity. This is slow but cheap and can achieve reasonably low moisture contents (particularly in dry areas).

Bigger seed takes longer to dry; seed with a high surface to volume ratio (for example, flat seed) dry faster than spherical seed. The presence of an aril, impermeable endocarp, or a testa provide considerable resistance to loss of moisture. Seeds with high oil content are easier to dry than starchy seed. The rate of drying will also depend on the thickness of the seed layer and the amount of air movement. For uniformity of drying, seed should be in thin layers and with sufficient ventilation. Because seed deteriorate rapidly at high temperature, ovens should not be used to dry seed.

Conditions of Storage

1. Long term (>25 years)

The preferred standards for long term seed storage published under the auspices of the Food and Agriculture Organisation of the United Nations (FAO) in 1975 prescribed seed storage at -18°C or less in air-tight containers at a seed moisture content of 5 +/- 1%. This standard was adopted by the then International Board for Plant Genetic Resources (IBPGR), now the International Plant Genetic Resource Institute (IPGRI), and the moisture content recommended was revised to 3-7%. This has been further amended to equilibrium conditions of 15°C-20°C and 10-15% relative humidity and frozen at -18°C or lower. Seed can be dried over silica gel or air dried in dry climates then stored hermetically or seed can be stored unsealed in a humidity controlled cold room in the open.

2. Medium term (5-25 years) and short term (<5 years)

If seed is to be distributed or used within the medium or short term, then storage can be at higher temperatures. Temperatures for medium term storage can be at 0-10°C with relative humidity of 15% or less. For short term storage seed can be held at room temperature or in air conditioned rooms. Nevertheless seed should be dry and free of insect predators and cleaned well. The storage period will have implications for the type of storage containers used in the genebank. (ie frequent access to the collection may be required for seed under short term storage conditions).

- Cool local conditions. If the seed has been dried and is properly packaged then storage in air-conditioned rooms can be quite effective. The room needs to be insect and rodent free.
- Domestic refrigerator or coolroom (4°C). Preferable to the above.
- Domestic deep freezer or freezer room (-18°C). Chest freezers are a cost-effective tool that will improve seed longevity. They are superior to upright models as they do not lose cold so quickly when opened. Upright models with multiple compartments and individual doors reduce this problem.

Containers

The type of storage (short, medium or long term) strongly influences the type of containers used. A wide range of containers can be obtained and vary in their ability to prevent moisture incursion. Whichever storage container is used, seed must be sufficiently dry before packing to prevent mould and fungal attack. Some possible containers are listed below:

- Laminated Aluminium Foil Bags are reasonably priced, but their quality varies dramatically. They require a heat sealer to close the bags and these come in all shapes and sizes. The bag is no better than its sealing. Pinholing can be a major problem with foil bags. An obvious advantage of foil bags is that they can be cut and resealed. Foil can also be obtained on continuous rolls or ready-made into bags.
- Glass Jars come in a variety of shapes and sizes and are limited by the effectiveness of their closures. They offer the possibility of checking for moisture uptake by including a colour indicator crystal within the jar. There is a slight risk of breakage if jars are dropped.

- Aluminium Cans also come in a variety of shapes and sizes and are limited by the effectiveness of their closures. The container should be seamless and have a good rubber gasket in the screw top. It is possible to put one container inside another (especially if the outer is glass and include a colour indicator to monitor any possible moisture changes).
- Plastic Containers are not advisable for long term storage as lids rarely fit tightly, but these may be used in the short term. Laminated plastic bags can also be used for short term storage and require a heat sealer to ensure moisture proofing.

Monitoring the Success of Storage

The success of seed storage depends on the longevity of the seed under the storage conditions used and the ability to regenerate adequate quantities of high quality seed without genetic change when required. A comprehensive monitoring program should be put in place at the outset of any genebank project. The determination of an appropriate on-going monitoring regime to assess response to storage is dependant on the quantity of seed collected and its original viability and moisture content. The majority of crop genebanks throughout the world monitor their accessions on a 5 or 10 yearly basis; others only periodically. Where new species are being taken into storage it is advisable to be conservative and monitor at more frequent intervals if seed supplies are available. Regular viability testing will determine whether seed is still healthy and alive. Don't wait until the resource has been lost in the wild before wondering whether your collection is still alive. A genebank should not become a genemorgue! Unfortunately, some holding institutions are fast becoming morgues as their stored seed loses viability and the rejuvenation and enhancement of their holdings lapse. Monitoring of the collection is a must, so that if viability declines steps can be taken to recollect or regenerate the collection. Regeneration for further seed collection will not an option for most of the species being dealt with, unless in the guise of translocations/reintroductions.

DOCUMENTATION

Genebanks are a resource centre for living plant material or germplasm and all information produced in genebank activities is of vital importance. Germplasm *per se* is of little worth in the conservation of genetic diversity without information on its identity, provenance and history since collection. A constant supply of accurate, reliable and up to date information is required for the genebank to function efficiently, whether the bank is a long or short term concern or whether the bank consists of living collections or seed. There is an obligation to record meticulously all field and laboratory data that represents that diversity. Attention to detail and a systematic approach is vital if the collection is to be useful. Comprehensive data files should be kept for all movement of material.

Documentation systems

Documentation systems range from manual card indexes and register books with minimal information, to complex computerised systems. Features of a documentation system include data integrity, fast information retrieval, user-friendly operation, flexibility and organisation. A documentation system or database is not only for

storage, but for data maintenance, processing, analysis and exchange. Computerised databases are an indispensable tool for the recording, analysing and retrieval of information, and provide for ease of accessibility and transfer of data. Hard copies of all information, including the original field data sheets, should nevertheless be kept on file and backups of computerised information should be made at regular intervals. Ideally, documentation should be standardised for the possibility of information exchange between germplasm storage facilities. Any changes in information (such as new identifications, distribution of seed etc.) can be recorded in the database with minimal delay.

In a seed-based genebank, the more information recorded about the seed collected and stored, the more valuable will be the accession for future use. All collections of seed will be assigned a unique accession number and all information from the field registered as soon as possible. The accession number is a unique identifier and remains with the seed throughout the processing, storage and monitoring phases and is the key to retrieval of information relating to the germplasm. All handling of the germplasm will be referenced according to the accession number.

1. Field Documentation

Provenance

Plant material of unknown origin has limited use for ongoing conservation research and management. Information should be sufficient to allow another person to return to the site and replicate an original collection. Point locality details (GPS latitude and longitude) are more precise data than kilometres on a map, but a GPS may not always be available. Roads change and kilometres on a map will too. Many old collections are located according to mile posts which no longer exist, making it difficult for later collectors to relocate the population. Be as accurate as possible with locational information.

Sampling Details

Whether a seed sample from the wild can be regarded as a statistically meaningful sample of the genetic stock of the population depends on the sampling techniques. For this reason, sampling details should accompany the seed. The collection date, collectors' name and collecting number are important aspects in sampling. Numbers of plants sampled will provide an idea of the genetic breadth of the collection and give an overall idea of what proportion of the entire population was sampled.

Supplementary data

Other data greatly enhance the conservation value of plants. A description of site characteristics, associated species, phenology, morphology of the taxon, condition of population and perceived threats is information that is highly recommended to record on a site visit. This is particularly important for rare and threatened species as some sites may become so degraded that little knowledge may remain of the original habitat. This may be vital information (for example, associated species, soil type and landforms) for future translocation projects and may point to possible new sites for the recovery of the species. These data may also give important insights into management problems that can be avoided. Information on the reproductive status of plants and productivity allow planning for future collecting trips.

Photographic records

A photographic record of the taxon and collection site is certainly desirable, and provides a visual record of the plant, its habit and its health. Habitat photos assist in relocation of a particular area and comparisons can be made between seasons and between years if a set photo point is maintained.

Voucher specimens

It is essential that specimens be accurately identified to species level. Vouchering of botanical specimens for lodging with relevant herbaria is of utmost importance. A specimen that represents the mean of the population is required. Multiple vouchers may be warranted when plants within the population vary. Voucher material from the source population must be retained to ensure that the accession can be verified and reassessed in the event of taxonomic reviews. This necessitates that the voucher specimen be numerically linked to the accession number.

2. Laboratory Documentation

Laboratory data for documentation includes germination/viability results, treatments used for germination, and moisture content/drying information (if applicable). Storage details and the regime for monitoring collections are also documented.

Germination Testing

Meticulous records are kept on the germination protocols attempted and the results. This will include the number of seeds tested, the number that germinated and when, the number of seedlings that appear abnormal and a detailed evaluation of the seeds that failed to germinate (hard, mouldy, soft, empty). Such information may suggest further germination procedures to attempt. For instance, scarification may be tried on seed that failed to imbibe, and firm seed may be subjected to further testing. The rate of germination may change over time and can be followed during monitoring of the accession in storage. A slower rate of germination as compared to a previous test may indicate genetic erosion.

Quantification for storage and monitoring

Knowledge of the exact number of seeds available within an accession is vital information. If seed is being used for distribution and for reintroduction accurate counts are essential. The type of quantification used will vary according to the fruit and seed type. Seed collected from small, easily extracted seed types (for example, seeds from the family Myrtaceae) are most efficiently recorded as the number of seeds per gram. To do this a small sub-sample is taken, counted, then weighed and the figure for the sub-sample extrapolated to the total weight of the collection. There will be instances when the seed cannot be totally cleaned from chaff (for example, many Myrtaceous species). In these cases, a set amount of the seed plus chaff is weighed and the number of seeds in that weight is counted then extrapolated to the remaining sample. Ideally 3-5 replicates are used for this type of quantitative assessment and the average of the replicates is taken. Other species warrant a careful seed count.

It is vital to know the numbers of seeds available in a genebank if the seed is to be used for future reintroduction projects or for research. All changes in the number of

seed available are noted accurately without delay. Monitoring in a seed-based bank establishes whether the seed amount and seed viability remain at acceptable levels, and whether a recollection or regeneration policy should be put in place.

Duplicate Collections

For safety of a conservation collection, it is advisable to send duplicate collections for long term storage to another institution that can hold these collections under the same conditions as the base. Monitoring need only be conducted at one institution. Depending on the worth of the collection it is advisable to put in place some form of formal agreement to ensure that in the long term, duplicate collections are not lost and remain the property of the original institution.

FURTHER READING

Brown, A.H.D. and Briggs, J.D. (1991). Sampling strategies for genetic variation in *ex-situ* collections of endangered plant species. In *Genetics and Conservation of Rare Plants*. Falk, D.A. and Holsinger, K.E., (eds.) Oxford University Press.

Cromarty, A.S., Ellis, R.H. and Roberts, E.H. (1985). *The Design of Seed Storage Facilities. Handbooks for Genebanks No.1*. IBPGR. Rome.

Ellis, R.H., Hong, T.D. and Roberts, E.H. (1985). *Handbook of Seed Technology for Genebanks, Vol. 1: Principles and Methodology., Handbooks for Genebanks No.2*. IBPGR Secretariat, Rome.

Ellis, R.H., Hong, T.D. and Roberts, E.H. (1985). *Handbook of Seed Technology for Genebanks, Vol. 2: Compendium of Specific Germination Information and Test Recommendations. Handbooks for Genebanks No.3*. IBPGR Secretariat, Rome.