

## **An Editorial Perspective on Seed Conservation**



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## Introduction

There is a broad spectrum of ideas on, concepts about, and approaches to, seed conservation. These have been articulated through various means of communication, including publication in this volume and in other media, public presentations and discussions. Many of these are the source of active discussion amongst seed conservationists. What follows is a brief editorial perspective of this broad yet fragmented ‘landscape’. It comes partly from a synthesis of the key points identified within the chapters, partly from the end of session discussions from the 2001 workshop, and partly from a review of very recent publications. Consequently, any views, where expressed, are those of the editors alone. A number of facts and figures are quoted that relate to information provided during the workshop discussions. This information is not found elsewhere in the book, is not sourced to a recent publication and should therefore be viewed as unverified. This chapter is meant only to serve as a quick reference guide to some current seed conservation foci. Each aspect should be considered in relation to the more detailed literature, particularly that contained within the previous chapters. Subjects are grouped according to the main sections of this book.

## Planning and Seed Collecting

- **Prior Informed Consent** (PIC) to collect seed or other botanical material at the national and local level is enshrined within the Convention on Biological Diversity (CBD) and, in many countries, national legislation. The CBD has encouraged the development of **Access and Benefit Sharing Agreements** (ABSAs) to help clarify what the parties involved with the seed collecting will get out of the exercise.
- **Species selection** for conservation depends primarily on conservation status and usefulness. Studies of the ecology and geography of species aid **population targeting**. These studies are assisted by information from, for example, local botanists, herbarium sheets, existing seed collections, floras, threatened status lists, and predictions using Geographical Information Systems (GIS). It should be noted that herbarium specimens provide a snapshot of historic distribution. They may have been sampled and collected for quite different purposes to that for which they are being used by seed conservationists. Furthermore, passport data for many existing collections is poor.

- Where **field survey work (and genetic analyses) prior to seed collection** is appropriate, the scale of the work will depend on the relative distribution of the species. Extensive sampling may be necessary to build an accurate picture of the distribution. Often, conservationists will not have the opportunities for such extensive studies prior to collection. In these cases, as many **population samples** from as many ecologically distinct sites across the range of the species should be collected, given the resources available. Even one sample per species is a good start to a conservation programme because it is likely that it will contain significant numbers of the known alleles of that species. **Collecting** should normally be random (selection of individuals) and even (from individuals) across the population and seeds collected in sufficient quantity to facilitate conservation without endangering the field survival of that population. ‘Randomness’ of collecting may be easier to recommend than it is to achieve. In contrast to most seed conservation work, forestry programmes often collect seeds from individual elite genotypes. Where warranted (e.g., small populations of very rare species), keeping maternal lines separate facilitates both maintenance of genetic integrity during multiplication and genetic analysis. However, this approach does increase curation effort. With regard to genetic analyses, taking a leaf dried with silica gel at the time of seed collection from each individual enables, through DNA extraction, the preparation of a ‘**genetic voucher**’. Whatever the sampling approach carried out, the method should be recorded with the passport data. Such **field data** should be as objective and extensive as possible, and geo-referenced. Collected samples can be used to inform further collecting.
- The genetic makeup of collections made over consecutive years almost certainly varies as a result of **changes in population structure** in the field. Furthermore, the **timing of sampling** during one season will influence genetic representation. Thus, where resources permit, better representation can be achieved by making collections from the same population twice in one season. Varying conditions from year to year will also affect the **physical and physiological status and heterogeneity of the collection** (size, colour mix, developmental spread and dormancy).
- Much work is needed to devise tests that can be used in the field **to screen for likely tolerance of seeds to drying**. It is probable that a suite of techniques will be needed.

- **Maximising seed quality at the time of collection and during transit** will significantly improve long-term storage potential. Extra effort to collect seeds at an appropriate stage of development, such that they tolerate rapid, enforced drying and banking, is a good long-term investment. For many species, the completion of seed growth, as measured by seed dry weight, generally coincides with **morphological changes** in the seed and fruit, for example, a colour change from green to red, yellow or brown. Wherever possible, dispersal units (seeds or fruits) should be collected when they are close to the **point of natural dispersal**. There is a high probability that the moisture status of dispersal units at the time of collection will be higher than, and not in equilibrium, with ambient levels. **Measurement of moisture status at the time of collection** with an appropriate hygrometer will inform post-harvest handling decisions. At the point of collection, the equilibrium relative humidity (eRH) of dispersal units and/or seeds may serve as an indicator of the stage of development and/or the risks associated with later drying. Seeds at a very high equilibrium humidity (> 95%) might be desiccation sensitive, whilst those naturally shed at a lower equilibrium humidity are more likely to be desiccation tolerant. Collections can be safely held in porous bags when the seed eRH and ambient temperature are such that the expected rate of deterioration would be acceptable (approx. 0.1 probits per month) or ambient RH is 50% or less. For all species, collecting technique, visual inspection and hand sorting can be used to separate dispersal units at different stages of maturity. It might be necessary to adopt different **treatments for each maturity class during transit**. Immature dispersal units might need to be held at high RH to encourage further ripening. In contrast, mature dispersal units might need to be artificially dried as soon as possible. Continued ripening of immature dispersal units can be achieved by holding uncleaned collections at a high RH under ambient temperatures in a partly-ventilated container. Great care must be taken to ensure that the dispersal units are aerated and that they do not become mouldy or begin to germinate. Transport of all collections from the field to the laboratory in unventilated containers, in a non-air-conditioned vehicle, risks overheating and seed viability loss. Furthermore, it should be noted that, although often unavoidable, transit of collections in the unheated hold of an aircraft risks chilling/cold stress for some tropical seeds. Additionally, there may be potential genetic effects through exposure to high altitude radiation and security screening devices. Such effects are likely to be small.

- Assessment of seed quality changes during development using [molecular] **maturity markers** is an attractive prospect, though application, at least in the short-term, is more likely to be in the laboratory rather than in the field. Embryo (axis) moisture contents can be used to indicate developmental age, but may require access to at least a 5-place balance (i.e., weighing to 10 µg). Simple monitoring of seed fresh weight can indicate the timing of mass maturity. A sharp decline signals the onset of the post-abscission phase when there is a high probability that seeds will survive seed bank processing. Chlorophyll fluorescence is now being used as an instantaneous, non-destructive method to characterise and sort mature seeds of a limited number of crops, e.g., tomato and white cabbage (Jalink *et al.*, 1998, *et seq.*). Interspecies differences in chlorophyll fluorescence may, though, limit the wider application of this technology to seed genetic resources.
- Field data is greatly enhanced by the inclusion of **indigenous knowledge** (IK). Making IK widely available, through formal publication, reduces the chance that commercial development can proceed without the holder of the IK having a stake. However, release of IK (and local botanists' knowledge) can be a sensitive issue, especially for specimen-related information e.g., the geo-location of IUCN Red List species of potential horticultural or medicinal value. Release of general information about the conservation status of species can be used positively with conservation authorities and the public. Such releases of information (in seed lists, newspapers, popular articles, books and papers) and their syntheses should be agreed with the original national and local stakeholders and preferably contained within an ABSA (see above). These agreements will also clarify whether the seed bank is able to distribute seed for use and what these uses may be. In many cases, a legally-binding **material transfer agreement** (MTA) between the bank and the user will be required, which retains rights over the collection on behalf of the national and local stakeholders. Such MTAs usually also control the passing of the germplasm to a third party.
- Where **re-introduction** is the immediate purpose of the collecting, adequate technical support, particularly for genetic diversity characterisation and in horticulture, will be required.
- Conservationists/conservation bodies are increasingly engaging **companies in the business of biodiversity** (see the guidelines in Earthwatch, 2002).
- Historically, the **exchange of germplasm** has benefited most, if not all, countries with respect to the use of exotic germplasm in agriculture (though perhaps not in relation to the introduction of **invasives**).

- Clearer guidelines on access to crop genetic resources are also becoming available, i.e., The **International Treaty on Plant Genetic Resources for Food and Agriculture** (ITPGRFA) which was adopted by the member states of the UN Food and Agricultural Organisation (FAO) on 3 November 2001 (Cherfas, 2002; [www.fao.org/ag/cgrfa/news.htm](http://www.fao.org/ag/cgrfa/news.htm)). The Treaty will enter into force after ratification by 40 countries. The Treaty aims to open up access to crop plant genetic resources (and associated information) and to ensure that benefits (financial, information exchange, access to technology, and transfer of technology) are shared equitably. The Treaty applies to 35 food crops and some 80 forages, with some notable exceptions, including soybean, peanut and some tropical forage species. It is intended that commercialisation of a resource under the Treaty would attract a royalty payment to help farmers in developing countries, who conserve and use crop diversity. It would appear that where their interests overlap, existing bilateral ABSAs are likely to have precedence over the ITPGRFA. However, for managers of some banks that supply both species covered by the Treaty and species not covered, and that also supply for both agricultural and non-agricultural use, there will be added complexity in organising seed distribution. This is most likely to be simplified by such seed banks developing two seed lists, one in relation to material with facilitated access covered by the Treaty and one for other material. Users of the former list would then appear to need to declare that they were from a country that had ratified the Treaty and that use was for the purposes of food and agriculture.

## Seed Processing and Testing

- All aspects of **processing can potentially have a negative impact on seed quality**.
- **Mechanical damage** arising during seed cleaning usually has a negative impact on longevity and can, in severe cases, reduce viability. However, mechanical damage of seeds possessing physical dormancy may improve germination.
- Whatever processing steps are used, it is essential to keep an **accurate record** of them.
- Because both **immature orthodox seeds up to a certain age and recalcitrant seeds are desiccation sensitive**, it can be difficult to separate them on the basis of their response to drying. Only

detailed developmental studies will allow their separation. Survival of desiccation is often dependent on drying rate. Rapid enforced desiccation in dry rooms that meet international recommendations (10–15% RH, 10–25°C) is tolerated in the case of mature (i.e., approaching the point of natural dispersal) orthodox seeds. Delayed or slow drying benefits immature orthodox seeds by permitting the continuation of development. In some species, e.g., *Millettia leucantha* Vatke, drying in the sun or shade did not reduce seed quality compared to a seed bank dry room. Nevertheless, the recommendation is still to **use drying facilities that meet international standards**, but when doing so to ensure adequate ventilation if seeds are to dry effectively.

- For **recalcitrant seeds and axes, rapid drying may lower the observable critical moisture content for survival**, although there may be an optimal rate of drying, with silica gel drying of axes being too rapid (Sun, 2002).
- Individual seed moisture contents are more meaningful, though more time consuming, than measurements based on bulked (many seeds) samples.
- The fields of **metabolomics and genomics** may provide insights as to why some seeds do not tolerate drying.
- Unfortunately, different research laboratories measure seed moisture status and express germination results in different ways, thereby making it **difficult to compare data**. For example, critical water content for desiccation sensitivity of a seed lot has numerous definitions (onset, endpoint and mid-point for viability loss), thus it is crucial to define how terms are being used when reporting data. Furthermore, there are differences in the way that different organisations and operators use terms such as ‘viable’. For bank testing, striving for consistency through time (potentially many decades or even centuries) within each bank is essential. Depending upon dexterity and other aptitudes, different staff may obtain different test results. Some quantification of these differences (e.g., with respect to hand chipping of seeds) can be illuminating.
- Due to imbibition damage, or where testing is protracted, **seeds can die during the germination test**. Imbibitional sensitivity is a neglected area of study in seed conservation science.
- Deeply dormant seeds tend to, but do not always, react to a viability stain such as 2-3-5 triphenyl tetrazolium chloride (**TZ**). Dead and infected seeds can give a false positive response to **TZ**.
- Knowledge of phylogenetic background, life form, habitat preference, regeneration strategy, distribution, and seed structure can be useful in **predicting seed dormancy type**.

However, the depth of any physiological dormancy can vary in time (within the year and between years).

- **Thermal scarification** can be used to remove hardseededness (physical dormancy) in dry seeds of some species, using many hours at 70°C or shorter periods at 100°C. However, whilst such treatments may have potential for treating bulk seedlots destined, for example, for restoration projects, they are not recommended for routine seed viability testing. It should be noted that site-directed thermal scarification of individual seeds is useful for such routine testing.
- Increasing the understanding of seed dormancy and refining the methods for its removal will be **key research priorities** in wild species seed conservation.

## Seed Storage and Utilisation

- The **seed viability equations** have been used to describe the storage performance of > 50 species over a broad range of environmental conditions (storage temperature and seed moisture content). However, predictions from the equation fall down for some species in a limited part of the sub-zero temperature range, not necessarily coincident with conventional seed bank storage temperature. Investigations on molecular mobility in relation to **viscosity** suggest that the viability equation might under- and over-estimate longevity with respect to certain storage conditions. Systems at the same viscosity do not appear to have the same longevity, suggesting inherent differences in longevity. In essence, this is represented in the viability constants.
- Optimum longevity appears to coincide with the lowest level of **volatile gas emission** at c. 20% RH. Very low RH treatments (i.e., ultra-dry) can be damaging to some seedlots. Others appear to tolerate well very dry (and cold) storage.
- At the beginning of 2003, it is still impossible to state categorically the number of species possessing orthodox seeds in relation to the total number of Spermatophytes (some 250,000–400,000 seed-bearing plants). Based on the study by Hong *et al.* (1998) of some 7,000 species, it seems reasonable to assume that **a significant majority of Spermatophytes, including many from the tropics, will have orthodox seeds**, but much work remains both to quantify the behaviour and to understand the differences between the three main seed storage types.

- **Key storage research subjects for the future** include:
  - optimisation of seed storage conditions;
  - rapid diagnosis of seed storage type;
  - improved understanding of ‘intermediate’ and ‘recalcitrant’ seeded species; and
  - refinement of storage techniques for intermediate and recalcitrant seeds.
- At the technological level, more work is required to increase **container effectiveness**, and on improving seed longevity, when using **cost-effective local alternatives to the more conventional drying and freezing facilities**.
- More attention should also be given to the **genetics of seed survival**. One working hypothesis is that seeds that die first within a seed population under poor storage conditions will be those that behave in a similar fashion under optimal conditions. In this fashion, placing seeds in a seed bank merely slows down the rate of loss of viability proportionately for individual seeds. This seems to be a reasonable hypothesis based on existing data, but little has been done to prove this, especially given improvements in DNA analysis. Should this hypothesis be true, then it becomes clear that time in storage is a selection factor. However, the effects of this are minimised by adoption of a high regeneration standard. In any case, seed longevity may also be acting as a selection factor in the field (though, admittedly, in many instances this may relate to longevity above the upper critical moisture content). Selection in storage has occasionally been quoted as a criticism of seed banking. In reality, such selection is probably minor in comparison to the effects of poor sampling in the field and poor regeneration practices. Furthermore, seed banks protect conserved samples from the continued evolutionary pressures that occur under *in situ* conditions. This means that samples may be poorly adapted, if grown back in the wild (e.g., re-introduced). The extent of this problem with respect to re-introduction, will relate to the generation time of the species and the severity of environmental change at the site from which the sample was taken. In some circumstances, such protection may be beneficial, e.g., when samples are required for monitoring genetic change at a site, or where the *in situ* populations are at risk of dramatic ‘genetic pollution’ from nearby crops. Although it might be argued that there are many imperfections in the genetics of seed conservation procedures, this must be balanced against the lack of suitable alternatives in many cases.
- **Partially dried recalcitrant seeds/embryos** tend to live short-term at ambient temperature, e.g., a few days.

- **Cooling rates** in the region of  $50^{\circ}\text{C s}^{-1}$  may be needed to reduce the likelihood of ice formation in relatively wet (c. 30% moisture content) recalcitrant seed axes being transferred to liquid nitrogen.
- Twenty year data for storage in liquid nitrogen dewars ( $< -150^{\circ}\text{C}$ ) and conventional conditions ( $-18^{\circ}\text{C}$ ) indicates some **benefit of ultra-cold (cryopreservation) storage**. However, this benefit needs to be considered against the increased technical difficulty of maintaining collections in cryopreservation.
- Sackville Hamilton *et al.* (2002) have recently considered the management of crop collections. Although **lumping and splitting of collections** may be appropriate for genetic and economic reasons, there can be dangers to seed longevity of combining collections of different age. Splitting population samples either at collecting (see above), or subsequently, has implications for curation effort. The need for dividing collections into **base and active** components is also being considered. While such divisions increase curation effort and therefore decrease efficiency, consideration of this matter by the Millennium Seed Bank Project led it to move away from a combined collection and back towards the maintenance of a rarely touched base collection and a more accessible active collection. The aim of this was to help maximise longevity in the base collection by reducing moisture uptake to an absolute minimum over long periods through very infrequent access, and by the use of double packing. Clearly, seed storage considerations should have a strong bearing on any strategy. By reducing the frequency of regeneration, costs and loss of genetic integrity are minimised.
- There are obvious merits in conserving **symbionts** as well as seeds, e.g., rhizobia and trees. Some institutes are involved in conserving associated microbes, e.g., orchid mycorrhizae at the Royal Botanic Gardens, Kew, and ericoid mycorrhizae at Kings Park and Botanic Garden, Perth, Western Australia.
- All plant genetic resource (PGR) facilities want to see an increase in **access to stored germplasm** and in some countries, e.g., Australia, such facilities are required to promote use.
- **Selection of germplasm for use** should relate to its provenance. GIS has a potential role in improving the use of germplasm appropriate to its site of usage. While the emphasis is often on use of genotypes collected near to where they are to be used, some data suggest that germplasm from a similar ecological background may be more important.

- The use of material from botanic garden seed banks may encompass re-introduction, trialling for agriculture or ecological restoration, or research (pure and applied). Due to the range of uses, **evaluation** for any particular purpose by such a bank is rarely feasible. Indeed, traditional crop bank evaluation of many agricultural traits may have limited value away from the trial site due to plasticity.
- Analysing the **flow around the world of PGR samples** is difficult as there have been about 1.7 million samples distributed over about 25 years. An apparent flow of samples to European institutes can, in some instances, relate to developing country scientists temporarily working abroad. About 10% of all world seed bank samples are held within the centres of the Consultative Group for International Agricultural Research (CGIAR). **CGIAR-related distribution of germplasm samples** is heavily in favour (4:1) of developing: developed country institutes. The total number of requests (for batches of samples) excluding 'repeats', is similar. About two-thirds of collections used are still within the respective CGIAR centres' conservation and utilisation programmes. Whilst there is free access to PGR databases and collections, private companies use them relatively little. Of those with a commercial interest, about 75% come from within developing countries. Even the bigger companies may only request about 50 samples per year. Around half the CGIAR collections dispatched are used by national agricultural programmes, and much of the rest is used by universities. Often such work is published in the scientific press. The percentage of CGIAR gene bank collections used at least once, varies with species, from around 35 to 94%. Availability of information on collections has major impact on use, e.g., ICRISAT (International Crop Research Institute for the Semi-Arid Tropics) sorghum collection requests fell after the general level of programme reports reduced after 1992. ICRISAT now takes a proactive role in spreading information on its collections. Nearly 50% of groundnut use programmes in India have involved accessions from ICRISAT. Generally, the number of requests for collections received by CGIAR centres has remained static. The pattern of distribution of a 'resource' with time since first made available varies from species to species, e.g., there has been an exponential decrease in requests for some sorghum lots. Multi-locational trials decreased in the 1990s as a result of the tightening of legislation on PGR distribution. Wider internet access will mean extra controls in relation to bilateral agreements and the ITPGRFA, and this may have a negative impact on the requests for seeds on seed lists. Funding constraints in national programmes has led to an increase in public-private partnerships for the testing and evaluation of collections, e.g., the

sponsorship by Pioneer Inc. of multiplication and regeneration of maize in South America. Although distribution data provide a measure of use, more information is needed on the ultimate impact of using the genetic resource.

- Re-establishment in the natural environment could involve regeneration and succession from the **soil seed bank**. Appearance of species from the soil seed bank in some areas may be stimulated by the application of heat (fire) and/or smoke-related chemicals as a liquid.
- Use is one area where *ex situ* **meets in situ conservation**. *In situ* conservation areas focus primarily on the landscape and tend to protect populations, the diversity of which may vary with time. Information management on and access to the areas can be poor, and they are prone to stochastic threats. In contrast, *ex situ* conservation programmes tend to protect populations, the diversity of which probably varies little with time (though genetic changes can occur through poor regeneration practice and as a result of excessive seed lot ageing). Information management on and access to such collections tends to be good.
- The long-term financial security of genetic resource collections (mainly seed banks) is a major recent concern. The CGIAR centres created 'Future Harvest', an organisation dedicated to building support for international agricultural research. The centres are thus now known as 'Future Harvest' centres. '**The Global Conservation Trust**' is a foundation bringing together FAO and the 'Future Harvest' centres, in an attempt to fund an endowment to secure key collections (see [www.startwithaseed.org](http://www.startwithaseed.org)).
- There is a massive human effort required to handle the quantity and quality of data in large **genetic resources databases**. Making databases operable in a network context requires the scaling-up, or combining, of smaller databases into an integrated whole. Enhancing information networks to the full requires collaboration between numerous, diverse partners, using **international data standards**. However, achieving data standards is difficult. Europe aims to increase data standards from c. 50% to c. 75% for standardised key fields in the next few years. The European Plant Genetic Resources Information Infra-Structure (EPGRIS) has been established to bring together collection data from across the continent ([www.ecpgr.cgiar.org/epgris/](http://www.ecpgr.cgiar.org/epgris/)). Network incorporation can be gradual; partners do not need to incorporate all fields initially. Public databases are free; those of companies have some form of restricted access. By way of comparison, it is worth noting that free access to sequences of the human genome held by Celera Genomics Corporation is

limited to 1 million base pairs per day. Access to the entire sequence requires a licence agreement and private sector access is subject to a negotiable fee (see Patrinos and Drell, 2002). Similar arrangements are in place for access to the rice (*Oryza sativa* L.) genome sequences held by Syngenta International. A key issue is to get companies to collaborate more with the public sector. Institutes in the public sector also need to balance the books and protect their intellectual property. Public sector scientists/institutes have always needed to 'publish or perish.' For large databases, the considerable up-front investment demands a new dogma of 'part-publish and prosper.'

- The **Global Biodiversity Information Facility** (GBIF; [www.gbif.org](http://www.gbif.org)) aims to enable exploration of the world's vast quantity of biodiversity information for environmental, social and economic uses. GBIF is developing a portal to provide specialised search engines for accessing digitised, geo-referenced specimen data. In the area of seed conservation, as research, processing and maintenance information becomes available within the seed-banking community, it is important that rapid dissemination takes place in order that time and effort is not wasted.

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