The management of the Kew Seed Bank for the conservation of arid land and U. K. wild species

Roger D. Smith & Simon Linington

... there is little glamour or recognition in the maintenance of collections of wild or weedy forms. ... sooner or later we will need to exploit all the germplasm that is within genetic reach.'

J. R. Harlan (1984)

Abstract

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The conservation work on wild species by the Royal Botanic Gardens, Kew, Seed Bank is described with particular emphasis on the differences between seed bank procedures for wild species and those for crops.

Introduction

Since 1974, the Kew Seed Bank at Wakehurst Place has been banking seeds of wild species for long-term conservation using internationally agreed standards (see FAO/IPGRI 1994). Currently, seeds are dried at 15°C and 15% relative humidity until they have reached equilibrium; they are then placed into containers and stored at -20°C (Prendergast & al. 1991). Seed banking is an appropriate technology for the conservation of many wild species (Smith 1985a, Prendergast & al. 1992) and this has been acknowledged in the Global Biodiversity Assessment (Heywood 1995).

Initially, the collecting targets of the Kew Seed Bank were focused on the *Gramineae* and *Leguminosae* that occur in the N.E. Mediterranean region. Since 1983, the primary target species have been those known to have human use in the arid and semi-arid tropics.

This target (identified by Kew's Survey of Economic Plants for Arid and Semi-Arid Lands, SEPASAL) contains some 2% of the world's Spermatophyte species, 11% of the accepted genera and 47% of plant families (see Davis & al. in press). They are spread throughout 10 of Takhtajan's 37 floristic regions of the world (Takhtajan 1986). To date some 4,000 collections from dryland countries are held in the Kew Seed Bank, representing about 600 species listed by SEPASAL.

In addition, the Kew Seed Bank holds 655 species from within the U. K., about 45% of the native species thought to produce seed. In total, the Bank holds some 9,943 collections banked from over 3,990 species representing nearly 200 plant families. The *Gramineae* and *Leguminosae* are the families most represented with 1,965 collections (557 species) and 2,463 collections (726 species) held respectively. The collections originate from some 106 countries. About 70% were collected directly from the wild and have not been the subject of regeneration.

Thus compared to crop seed banks, the Kew Seed Bank's strength is in the genetic width rather than depth conserved (i.e. interspecific rather than intraspecific variation). The experience gained from handling these collections and the peculiar difficulties associated with the curation of wild plant germplasm are given below.

Availability of information

In relation to the estimated 240 000 seed-bearing plant species known to science (Mabberley 1987), comparatively few species have had their seed storage physiology studied. A recent survey (Hong & al. in press) revealed information on just 6,866 species, though storability of some additional species might be deduced by, for instance, reference to their sale in trade. Within this limited database and in comparison with cultivated species, little is known about the biology of wild species at any level. For example, when a bibliographic search was made to discover the seed storage physiology of genera on the SEPASAL database, nothing was known for two in every three genera. The same is also true for seed dormancy and other characteristics useful to genetic resource managers such as growing requirements and breeding system.

For example, in the family *Labiatae*, of the 221 genera and 5 600 species (Mabberley 1987), only 50 genera and 368 species have had their breeding systems studied (Owens & Ubera-Jiménez 1992).

In comparison with managers of crop gene banks, those working with wild species will need to know enough seed physiology to extrapolate with care from well known species through the scarcely known to the unknown, or be supported by an active seed research group. Within Kew's Seed Conservation Section, both approaches have been adopted and we believe them to be the cornerstone of our success in seed conservation.

Seed germination testing is deliberately delayed until after seed has been dried and frozen so that a positive result establishes both that the species is desiccation-tolerant and that long-term conservation is possible.

This is necessary, as research at Wakehurst Place has established the possibility of all seed storage types occurring within the same genus (Dickie & al. 1991, Tompsett 1994). The seed conservation activities serve, therefore, also as a rapid screen for seed storage behaviour.

Seed regeneration

The lack of basic knowledge about growing requirements of wild species, coupled with the fact that seed banks are often located in different environments from those where the collections were originally made, makes seed regeneration less than certain. Analysis of the successes and failures of attempting to regenerate Mediterranean germplasm under mesic U. K. conditions revealed it to be difficult, expensive and far from certain. Indeed, regeneration can often cost more than recollection from the wild (Thompson & al. 1981). At worst, the efforts of making representative collections in the field can be totally negated by the selection pressures experienced when the plants are grown well away from their natural habitats. Therefore the development of the Kew Seed Bank has concentrated on making large, high quality collections in the field and maintaining their viability through subsequent transportation to the bank and the processing of the seed. This wild-collected seed is then made available for use by means of a seed list, after it has been identified, its germination determined and its high viability confirmed (Linington 1994).

No regeneration takes place until seed numbers have fallen to low levels through use, thus allowing considerable savings of seed, human & financial resources until the value of the material has been better assessed (Linington & Smith 1987). These savings can then be used to improve the success of the seed regeneration which will be still be necessary for those species which produce low seed numbers or those which occur in small populations in the wild. Within the U.K. flora, low seed production is frequent only in shaded habitats. In the other habitats studied by Salisbury (1942), the average seed output for each species was in the range 1,600 - 3,200 seeds per plant. It is clear that even if only 20% of the seed is taken so as to ensure survival in the wild, large seed numbers (e.g. several thousand) will accrue if up to 70 individuals (to have a good chance of gathering alleles at frequencies as low as 0.05) are sampled. Where seed numbers are likely to be very low, Kew advises its collectors to keep the seed from each mother plant separate in order to maximise the genetic potential during multiplication.

Seed longevity

Current predictions indicate that many crop species can be stored a very long time in seed banks with little difficulty. For instance, using the predictive models of Ellis and Roberts (1980) and assuming the universal temperature constants of Dickie & al. (1990), barley seed dried to equilibrium with 15% relative humidity and 15°C, sealed in a container and placed at -20°C will fall in viability from 97.7% down to 84.1% in 688 years. Similarly, figures for Sesame (*Sesamum indicum*) and Sorghum (*Sorghum bicolor*) are predicted to be 1,332 and 6,106 years respectively (see Hong & al., in press, for a more comprehensive list of predictions). While seed longevity in many wild species is estimated to be considerable, for others stored under identical conditions to those above, figures can be as low as 32 years as for *Ulmus carpinifolia* (Tompsett 1986, Dickie & al. 1990). Due to this variability in wild species' longevities, it will be necessary for seed banks storing wild species to work harder to achieve the same overall longevity goals as those storing crop species. Surprisingly, no direct comparison has yet been made between the seed storage characteristics of any crop and one of its wild relatives.

For genetic resources managers the implications are clear. If seeds of wild species are to be maintained with equal certainty as those of crop species, their storage conditions will need to be more precise and their post-harvest treatment more considered. If not, the losses in seed longevity will negate the efforts made to collect them. The easiest way to compensate for the greater rates of loss of viability amongst wild species is to ensure the seed is put into storage when its storage potential is at its highest (Smith 1984, Smith 1995). When these conditions are met, the available data support the belief that storage lives of 100-200 years can be achieved for many wild species.

With a few exceptions, there is a noticeable lack of information regarding seed quality and maturation for both crop and wild species (see however Smith 1985b, Pieta Filho & Ellis 1991, Hay & Probert 1995). In crops with dry dehiscent fruits, seed maturation is accompanied first by the acquisition of the ability to survive rapid drying at low relative humidities, then by an increase in seed storage potential which peaks and falls away as the ageing of the air-dried seeds begins on the plant in the field. For fleshy-fruited crops, the processes are the same except that seed ageing does not occur in the fruit (Dickie 1988, Demir & Ellis 1992). In wild species, the patterns among the different fruit types appear to be the same though in the case of foxglove, *Digitalis purpurea*, a tailing off of viability is not apparent (Hay & Probert 1995). However, unlike crop plants, where seed shed has been prevented through selection, seed dispersal in wild species can take place before maximum seedlot storage potential has been achieved. In many crop plants, especially cereals and pulses, there has also been selection, for uniformity of seed maturation. This is in contrast to wild species where the seed is likely to be relatively heterogeneous due to the of flowering dates.

Although conventional seed bank storage of wild species has been studied in few species relative to the size of the plant kingdom, liquid nitrogen storage, used by some crop seed banks, has been examined in far fewer (300) species (Pritchard 1995).

Empty and insect-damaged seed

Seedlots of wild species can contain many empty or undeveloped seeds as well as insect-infested seeds (Linington & al. 1995). The frequency of these problems, which remain after seedlots have been cleaned, is assessed at the Kew Seed Bank by X-ray analysis. Examination of data gathered by this process reveals that the problems are widespread. Empty seededness was examined in over 4,000 accessions from 20 families and found to occur with frequencies of between 10-73%. In some families, particularly the *Ericaceae* and *Rhamnaceae*, the percentage of empty seeds per accession was found to have an average value as high as 10 and 30% respectively. Insect damage is also widespread but damage occurs in fewer individuals per accession. Failure to account for these variations will lead to uncertain viability monitoring which is likely to result in the unnecessary expense of premature seed regeneration.

Seed dormancy

The survival mechanism which prevents whole seed populations from germinating simultaneously when the minimal requirements for seed hydration and temperature are met, is known as seed dormancy. In crop species, such behaviour prevents the rapid and uniform establishment of the crop and is so selected against, during the early stages of domestication. In contrast to their crop counterparts, curators of wild species' collections will regularly encounter seed dormancy. On behalf of their customers, curators will need to identify the conditions which overcome seed dormancy if the efforts to make representative collections are not to be undone through the non-contribution to the next generation of the dormant genotypes. Surprisingly, no study has yet been undertaken to identify whether seed dormancy is tightly linked to any other genetic traits.

Within a species, the depth of seed dormancy varies greatly between seedlots, being an integration of both genotype and the environment under which the seed developed and matured as well as its post-harvest history (Probert & al. 1985a, b). However, the basic mechanisms of dormancy loss are probably the same within a species. Analysis of past Kew Seed Bank testing results suggest that similarities in the conditions that break seed dormancy can be found even at the family level. These analyses have allowed the development of schemes which can rationalise seed testing (in Ellis & al. 1985). The basic premise of these schemes is that firstly, users of germplasm will have few germination facilities available to them and secondly, that the schemes must also arrive at this answer using the smallest number of the banked seeds. Consequently, for each collection, the simplest conditions must be defined under which full germination takes place using the smallest number of tests. To this end, the probabilities of each condition giving full germination for all accessions is identified and the methods are then ranked for success. From such an analysis, it is clear that seed dormancy presents greater problems in some families (e.g. *Chenopodiaceae, Compositae* and *Gramineae*) than others (e.g. *Cruciferae* and *Leguminosae*). Moreover, particularly difficult genera can be found such as *Eragrostis* and *Panicum* within the *Gramineae*.

Finally, it should be noted that improvement of the storage potential of seeds arriving at the bank may increase the depth of seed dormancy encountered.

Plant identification

The identification of wild plants to the species level is considerably more difficult than in crops. However, the value of accurate identification, allowing curators and users to link more certainly to the bibliographic record is greater. For this reason, considerable efforts are made by Kew Seed Bank's collectors to gather herbarium specimens representing the mean of the population sampled. Following identification by a specialist, the herbarium collections are kept available for reference. Where voucher specimens are not available at the time of collection, plants are grown from the seed at Wakehurst Place to provide them. This situation usually results from the fact that when seeds are available, flowering may well be complete. Under these circumstances there is also the increased risk of a mixed collection if closely related species are sympatric.

Plant health

Whilst acknowledging the risks of importing novel pests and diseases with the seeds of previously unstudied wild species, the difficulties of taking appropriate action are enormous. Both the legislation and the research literature are necessarily highly biased towards the major agricultural crops. The expense and frequent impracticality of undertaking seed-to-seed plant health monitoring represents a major difficulty in meeting conservation objectives and responsible use of wild plant germplasm. The value of strict control over unwanted plant fragments which remain after seed cleaning cannot be over-stressed. At the Kew Seed Bank, all such plant parts are destroyed either by incineration in sealed containers or through solvent extraction prior to incorporation in chemical screens. Interaction with the U.K. plant health authorities has resulted in better practice without impeding germplasm availability.

Evaluation

The wide range of users that request material from the Kew Seed Bank (from blood transfusion units, through ozone pollution researchers to those undertaking trials of living fences in developing countcries, as well as the more usual users of plant genetic resources) prevents a cost-effective evaluation programme from being established. However, it is hoped that the use of geographical information systems (G.I.S.) together with the high level of spatial referencing of the Bank's collection will enable more directed usage of the wild germplasm as well as improved collection planning.

Seed distribution

The level of requests made for material held by the Kew Seed Bank is relatively high in comparison to that made for material stored in major crop gene banks. The frequency of request for each item offered in the 1992 Kew List of Seeds was 0.34. This has implications to the type of storage container used by the Bank, because frequent access to the collection is required. A variety of different openable containers are used. As with all containers, openable or not, none is perfect and substantial research is required in this neglected area of long-term conservation.

Costs

One factor often quoted as negating the applicability of all seed banks in conservation strategies is their cost. A recent analysis of the costs of Kew Seed Bank activities shows that once the bank facility is established the main costs are the collection and processing of the material into the store. The full cost of collecting each population sample from overseas is close to £350 and incorporation into the bank (including initial germination testing, cleaning, packaging and verification) is about £160. Subsequently, annual curation costs per sample are £3 and distribution costsbout £9. Crop seed bank unit costs are likely to be lower for the reasons outlined above. We would argue that all seed bank costs are low for the certain underwriting of the continued survival of plant populations and compare favourably with the full costs of *in situ* conservation.

Conclusions

The experience of 20 years of banking the seeds of wild species can be summarised as follows:

(1) Providing the seeds are desiccation-tolerant, storage lives under currently accepted standards should be sufficient (100-200 years) to achieve valuable *ex situ* conservation goals.

(2) To achieve these goals, curators working with wild species will need to be more careful in their working methodologies than their crop counterparts.

(3) These methodologies can often be extrapolated successfully from those used in crop seed banks provided the problems associated with seed regeneration, quality, dormancy and health are given proper attention.

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Address of the authors:
Dr. R. D. Smith & Dr. S. Linington, Seed Conservation Section, Royal Botanic Gardens, Kew, Wakehurst Place, Ardingly, West Sussex RH17 6TN, U.K.