The Millennium Seed Bank Project and its Approach to Germination Testing©

Robin Probert
Seed Conservation Department, Royal Botanic Gardens, Kew, Wakehurst Place, Ardingly, West Sussex, RH17 6TN UK

INTRODUCTION

The Millennium Seed Bank Project (MSBP) was instigated as a means of addressing the large-scale loss of plant biodiversity. Human numbers have increased two-fold since 1960 and the United Nations predicts a further 50% increase by 2025. Land conversion for development and agriculture leads to an inevitable loss of biodiversity for there is a direct relationship between species numbers and land area available. Over the last 400 years, the extinction of 584 plant species has been recorded, most of them in the last 100 years. This rate is 70 times faster than expected from the geological record.

AIMS OF THE MILLENNIUM SEED BANK PROJECT

Through a network of international partnerships, the project seeks to conserve wild plant species and make their seeds available for research and sustainable use. Based at Wakehurst Place, West Sussex, it was conceived, developed, and is managed by the Seed Conservation Department of the Royal Botanic Gardens, Kew. Its principal aims are to:

Conserve UK flora by the Year 2000. Working closely with the statutory bodies English Nature, Scottish Natural Heritage, and the Countryside Council for Wales; numerous Wildlife Trusts; Botanical Society of the British Isles (BSBI) and the general public, 93% of the UK's seed-bearing plants have been successfully collected and banked. Work will continue to secure the remaining bankable species and to increase the genetic representation of those that are most threatened.

Collect and Conserve 10% of the World's Flora. Some 24,000 species will be collected, principally from the drylands, by the year 2010 (see International Programme, below).

Solve Seed Problems. Unlike the seeds of crop plants, seeds of wild plant species are generally less easy to germinate because of the presence of dormancy. There is also wide variation between species in potential longevity and some are inherently difficult to store. These are the principal challenges for project's research scientists.

Transfer Technology. The project seeks to build capacity for seed conservation among its international partners through training and the transfer of technology and information.

Promote Sustainable Development. Under the terms and conditions of Material Supply Agreements, samples of seeds are made available for utilisation and research.

Educate and Involve the Public. The project educates the general public on the importance of conservation and highlights the role of the Millennium Seed Bank. This is achieved through a public exhibition that sits in the heart of the Wellcome Trust Millennium Building.

INTERNATIONAL PROGRAMME

The central thrust of the project is the development of bilateral partnerships with countries that possess extensive areas of dryland. Drylands cover a third of the Earth's land surface, including many of the world's poorest countries, and support almost one fifth of its population. The most immediate threat to dryland areas is desertification, exacerbated by intensive human settlement. Plants that inhabit the drylands are particularly well suited to ex situ conservation using seed bank technology.

RBG Kew is determined to honour the letter and spirit of the Convention on Biological Diversity (CBD), the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), and other international, regional, national and subnational laws and policies concerning biodiversity. RBG Kew is wholly committed to the principles of Prior Informed Consent (PIC) and mutually agreed terms.

Before work can begin in any of the partner countries, Kew scientists must work closely with overseas collaborators to define scientific goals, activities, and outcomes. These project documents are bound within legally binding Access and Benefit Sharing Agreements (ABSAs) that are negotiated, often at governmental level, between legal representatives.

MSBP Partners. By 1 November 2001, the MSBP had successfully negotiated ABSAs with 12 countries or states:

Africa. Burkina Faso, Egypt, Kenya, Madagascar, Namibia, and South Africa

Americas. Chile, Mexico, and United States

Asia. Jordan, Lebanon

Australasia. Western Australia

Project development is also underway in Botswana, China, Ethiopia, Malawi, Morocco, Tanzania, Tunisia, Saudi Arabia, and Uganda

Main Activities of Collaboration.

Joint Seed Collecting. Collecting priorities are largely determined by partners, advised and supported where appropriate by Kew scientists. In all cases emphasis tends be placed on species represented by the so-called three Es: Endangered, Economic, Endemic.

Providing Safe Duplicate Storage at the MSB. This is the vital "insurance policy" offered by Kew to its partners. Under mutually agreed, legally binding terms, duplicate collections are held in trust at the Millennium Seed Bank. Secure long-term funding, and state-of-the-art scientific and technical backup are obvious benefits.

Training and Capacity Building in Seed Conservation. The project offers a flexible mix of training opportunities for partners ranging from informal, technical on-the-job training to in-country, focused training workshops. Depending on partner needs, training is offered across the full spectrum of academic attainment from technician to post-doctoral researcher.

Joint Research. Research scientists from partner countries have access to the project's research laboratories where they can study problems arising from their conservation programmes working on seed collections from their country of origin.

Information Exchange and Dissemination. Information and knowledge is shared freely between partners.

Encouraging Opportunities for Habitat Restoration and Local Utilisation. Distribution of genetic resources for research and utilisation is one of the tenets of the Convention on Biological Diversity. The project works with its partners to ensure that the seed collections are used, particularly in the country of origin, to improve the livelihoods of local people.

SEED PROCESSING ACTIVITIES AT THE MILLENNIUM SEED BANK

Drying. Fully mature seed collections are dried as soon as possible after harvest. At the MSB seeds are dried at 15% relative humidity (RH) and 15°C in drying rooms controlled by lithium chloride rotary dryers. Under these conditions, seeds lose water by desorption reaching an equilibrium moisture content of around 5% (fresh weight basis) in about 3 to 4 weeks. This reduction in moisture content from levels in equilibrium with field conditions (approximately 80% RH) increases the subsequent shelf life of the seeds in the seed bank by as much as 1000 times. As a general rule, seed life spans double for every 1% reduction in seed moisture content.

If seed collections are not fully ripe, they may be held initially at ambient conditions allowing gentle drying and further ripening before transfer to the main drying room.

Cleaning. Because of the considerable diversity in seed structure and morphology, all seed processing is by hand. Gentle rubbing using rubber bungs and sieves ensures that seeds are not damaged. A range of aspirators are used to winnow collections to remove debris and any empty, poorly developed, or damaged seeds and as a final check of purity a sample of seeds is X-rayed. This X-ray analysis is particularly effective in the detection of insect infested seeds.

Sealing. When seeds have been cleaned and re-dried they are sealed in an appropriate air-tight storage container. A range of containers are used at the MSB depending on the size of the seeds and the total volume of the collection. Containers chosen after extensive trials include glass bottles with plastic screw tops and preserving jars with clamp down lids and rubber seals. To provide a visual check that containers remain air tight during long-term storage, 1 g sachets of silica gel with blue cobalt chloride indicator accompany the seeds. A blue to pink colour change will indicate moisture ingress and the need to re-dry the collection and transfer to a new container.

Banking. Sealed containers of seeds are stored in the seed bank at -20°C. As with drying, reducing the temperature from ambient levels to -20°C also leads to a considerable increase in seed longevity. As a general rule seed life spans double for every 5°C reduction in temperature. Based on sound empirical evidence, mathematical modelling, and the viability of ancient seeds at archaeological sites, the MSBP has set a target for the majority of its collections to remain viable for at least 200 years.

Testing Seed Viability. The viability of the MSB's collections is periodically checked by germination tests. The current retest interval is 10 years. Recent 20-year retests on large numbers of collections banked before the start of the Millennium Seed Bank Project, as part of Kew's existing seed conservation programme, show for

the vast majority of cases no detectable decline in viability since the time of banking.

As a general rule, plain water agar at 10 g·litre^{-1} is the preferred substrate for germination testing at the MSB. There are at least three distinct advantages of using this medium: imbibition injury, which can occur when very dry seeds absorb water too quickly, is minimised when seeds are sown on agar; agar is very easy to prepare—simply dissolve in boiling water—and dormancy breaking chemicals or hormones, such as, gibberellins, can be readily incorporated; agar is relatively cheap compared to other substrates such as filter paper.

Seed Dormancy. Dormancy, common in seeds of wild plant species, may be defined as a failure of viable seeds to germinate under conditions usually favourable for germination. If dormant seeds are not recognised as such and are assumed to be dead then the true viability of a seed bank collection will be underestimated possibly leading to unnecessary regeneration, or even worse, premature disposal. The development of practical treatments for the removal of seed dormancy is thus one of the most important tasks for the project's scientists. Put simply, there is little point in conserving seeds unless there is a means of converting those seeds back into plants.

Table 1. Example of the principal types of seed dormancy encountered by the Millennium Seed Bank Project.

Dormancy type	Occurrence	Treatment	Examples
Physiological	Usually endospermic seeds with small embryos	Often warm or cold stratification; sometimes surgical treatment to expose embryo	Apiaceae, Iridaceae, Liliaceae, Papaveraceae, Ranunculaceae
Physical	Usually non-endospermic seeds. Presence of hard often impermeable seed coats	Scarification of the seed coat by filing or chipping	Cistaceae, Fabaceae, Geraniaceae, Malvaceae Rhamnaceae

Typical MSBP Seed Testing Protocol. The basic aim of seed testing at the MSB is to assess the true viability of a seed collection using the simplest conditions possible so that end users can reproduce those conditions without the need for sophisticated facilities. Thus a step-by-step approach is used with dormancy-breaking factors added only if required (Fig. 1).

Family Germination Schemes. The stepwise approach to germination testing at the MSB has enabled the development of family germination schemes that are used to guide the approach to seed testing at the family level. In these schemes, Stage I usually represents the basic conditions that have been found to be appropriate for non-dormant collections. However, for families where dormancy is so widespread that there is a high probability of dormancy in all collections (for example Fabaceae), Stage I will include the application of a dormancy-breaking pre-treatment. The following are examples of three family germination schemes:

***Asteraceae* (Compositae)**

I Constant temperature: 10, 15, or 25°C
II Alternating temperature: 25/10 or 35/20°C
III week at best temperature from (1) or (2) above; sterilise, excise embryo, incubate at 15°C
IV Cold stratification (seeds): 8 weeks at 5°C
V Apply chemicals: nitrate (0.1 g·litre^{-1}); gibberellin (GA_3 0.25 g·litre^{-1})
VI Tetrazolium test

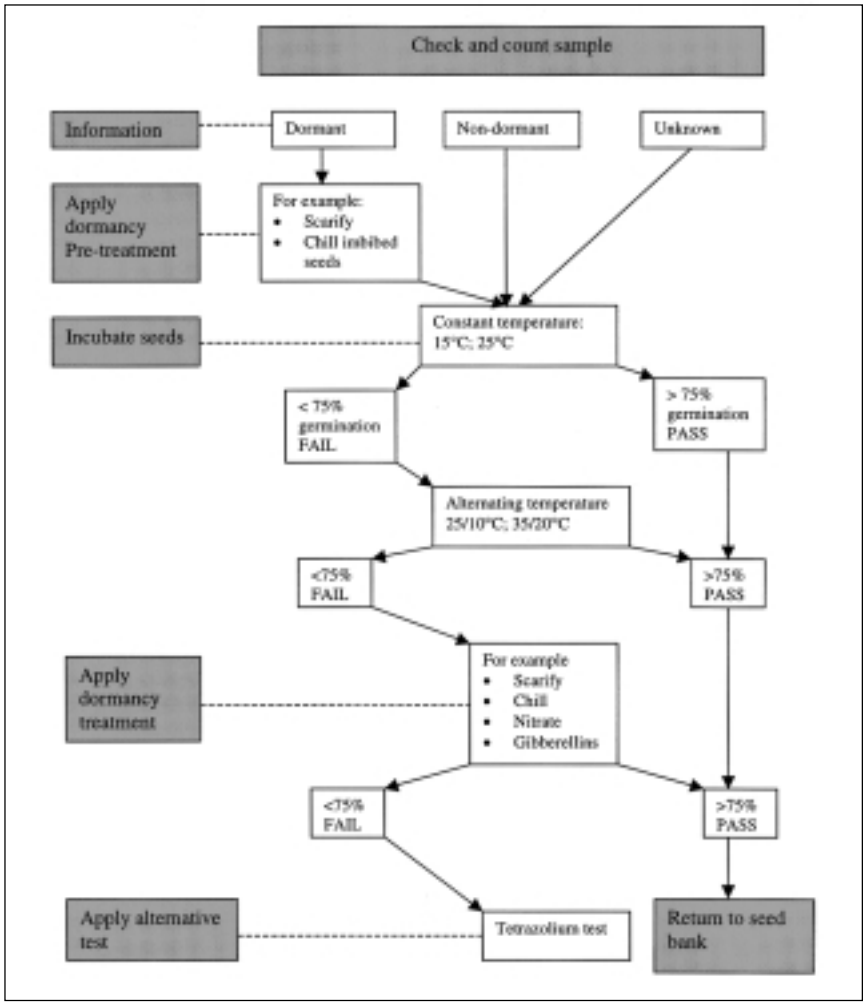

Figure 1. Millennium Seed Bank seed testing protocol.

***Fabaceae* (Leguminoseae)**

I Scarify (chip or file)
II Constant temperature: 15 or 25°C
III RH condition: hold seeds at 100% RH for 2 to 4 days at 20°C
IV Tetrazolium test

Liliaceae

I Constant temperature: 15 or 25°C
II Cold stratification: 8 weeks at 5°C
III Surgery: excise tissue to expose embryo
IV Tetrazolium test

INFORMATION

Information on the biology of seeds, generated by the MSBP and derived from other sources, is being complied into a seed information database (SID). So far, information on the storage behaviour of more than 7000 species has been compiled and the database is now freely available to be searched via Kew's website: <www.rbgkew.org.uk/data/sid>

In the near future the database will be widened to include other information such as seed weight, dispersal mechanism, seed anatomy/morphology, and germination/dormancy. Eventually holding information for many thousands of species, this will be the most comprehensive database available for seed information.

Acknowledgement. Generous financial support for the Millennium Seed Bank Project has been received from the Millennium Commission, the Wellcome Trust, and Orange plc.