

Chapter

17

## Processing and Maintenance of the Millennium Seed Bank Collections



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### Summary

This chapter contains details of the processing of seed material into the Millennium Seed Bank and its subsequent maintenance. The problems encountered in cleaning a diverse array of seed material are emphasised.

## Introduction

The Millennium Seed Bank (MSB) Project is an international conservation activity involving many countries. It is managed by the Royal Botanic Gardens, Kew, and receives substantial sponsorship from the UK's Millennium Commission, the Wellcome Trust and Orange plc. A key aim of the project is the conservation of seed samples of 24,000 species, principally from the tropical drylands, by the year 2010. Duplicates of most of these will be stored in the MSB of the Wellcome Trust Millennium Building (WTMB) at RBG Kew's satellite garden of Wakehurst Place in West Sussex, UK. The MSB is the successor of the Kew Seed Bank established at Wakehurst Place in 1974.

When processing such a diverse range of species, it has been necessary to adapt standard seed bank procedures, developed originally for crops (see for instance, Ellis *et al.*, 1985). This has been especially true for seed cleaning, where a wide range of seed and fruit morphology and structure is encountered.

The procedures are those shown in Figure 17.1 of the Introduction to this book. Most of the stages following collection are examined briefly in this chapter and special attention is paid to adaptations that have been found to be particularly useful. However, some activities are specifically covered elsewhere in this book: collecting (see Way, 2003 – Chapter 9); recording collecting and processing data on the Seed Bank Database, SBD (see Bone *et al.*, 2003 – Chapter 18); seed moisture status monitoring (see Probert *et al.*, 2003 – Chapter 20) and packaging (see Manger *et al.*, 2003 – Chapter 34). This chapter complements the account of forest seed processing by Schmidt and Thomsen (2003 – Chapter 16) within this book.

## Processing Stages

### 1. Shipment and Unpacking of the Seed-lots

Seeds are unpacked immediately upon arrival. This is done within a clean (and relatively contained) area, in case live insects are present. Collections are checked for signs of damage (for instance that caused by insects) and to assess whether immediate cleaning is required, as with wet fruits. Species' likely storage characteristics are decided by reference to Hong *et al.* (1998) and the Seed Information Database (see Bone *et al.*, 2003 – Chapter 18). Those that might pose storage problems are removed from the batch at this stage for drying tests, to establish desiccation tolerance. Any seed-lot containing live insects is separated for further treatment, consisting of drying for one week in sealed cloth bags and is then placed at -20°C for one week in plastic bags, before processing as normal. Collections which are required by UK Plant Health control to receive special isolation and which have restricted use, are kept separate from the remainder of the batch and processed according to agreed standard operating procedures. The majority of collections are placed in the drying room (see later) immediately, provided they are in sealed cloth or non-waxy paper bags. Seeds arriving in plastic or foil are repacked accordingly prior to drying. Herbarium specimens that accompany the seed material are also dealt with at this stage. That process is described later in the chapter.

### 2. Seed Cleaning

The purpose of the MSB is to conserve seed samples from wild species. Consequently, the dispersal units received at the MSB vary considerably in their structure and morphology. Accessions may arrive as cleaned seeds, uncleaned seed-heads or fruits (all broadly termed here as seed-lots or seed collections). They may have been cleaned in the country of origin, or arrive exactly as they were collected. Seed sample sizes may be small compared to many crop genetic resource collections, or those encountered in commercial operations. Therefore, seed wastage must be minimised. Seed collections need to be cleaned for several reasons:

- There needs to be reasonable reduction of plant bulk by extracting the smallest storable entity, usually the seed, without incurring any damage to the collection.
- Removal of unnecessary plant matter reduces the risk of disease carry-over.

- Empty and/or insect-infested seeds are removed where possible from the seed collection, as these ‘incompetent’ seeds complicate the interpretation of germination results and quantity determination as well as adding unnecessary bulk.
- Cleaning removes contaminants of the collection, especially seeds of other species or inert matter.

All cleaning is done by hand, with the aid of some simple pieces of equipment. Automated cleaning can give a degree of seed damage that may lead to loss of viability (see for example, Saini *et al.*, 1982). This is unacceptable for conservation collections. Automated processes are not flexible enough for the diversity of material handled by a seed bank conserving wild species. In addition, there is danger of cross contamination, only avoided by considerable cleaning of the equipment between collections.

Several methods are used to clean incoming MSB seed-lots. Wet, fleshy fruit material received at the bank is dealt with immediately by squashing it through sieves to separate out the seeds, followed by a tepid water wash. The extracted seeds are then spread in a thin layer and placed in the drying room (see section on ‘Drying, Packaging and Cold Storage’). Where a mucilage layer covers the seeds, drying enables it to be removed easily by gentle hand rubbing.

Four main techniques are used in seed cleaning of dried material:

- 1) *Gentle crushing/grinding using a rubber bung and sieve of suitable mesh size* (separation of seed from bulk material). This is the starting point for the cleaning of many collections. It is particularly suitable for species where the seed is relatively easily separated undamaged from the dried and crushed seed head. Such species include many of those in the *Boraginaceae*, *Lamiaceae* (*Labiatae*) and *Poaceae* (*Gramineae*). Material is checked under a binocular microscope to ensure that the rubbing is not harming the dried and therefore quite brittle seeds.
- 2) *Sieving with sieves of different mesh sizes* (further separation of seed and debris). Sieving using a column of sieves with different mesh sizes often follows the previous technique. Debris and dust is usually substantially removed from the seed-lot by careful manipulation of mesh sizes. With the collections of many species that have dry, dehiscent capsules, such as many of the *Campanulaceae*, *Liliaceae* (*sensu lato*), *Papaveraceae* and *Scrophulariaceae*, crushing is neither necessary nor desirable as the seeds are easily shaken out on the sieve. Furthermore, debris from the crushed head can be of similar size and weight to the seeds. The latter makes aspiration (see below) difficult. It should be noted that both grinding and sieving can liberate large amounts of dust. To protect workers, these cleaning actions are carried out in dust-extraction cabinets.

- 3) *Density separation using a seed aspirator* (removal, using a column of air, of lighter or heavier elements from collection, i.e., debris or empty or infested seeds). This stage usually follows sieving. The aspirators can cope with a wide range of seed sizes, from small seeded *Poaceae* (*Gramineae*) to some of the larger-seeded *Fabaceae* (*Leguminosae*). Airflow settings are recorded, so that the same conditions can be applied to similar collections in the future.
- 4) *Hand sorting* (individual, piece by piece removal of debris, *etc.*). A microscope or illuminated magnifying lens may often be essential for this work, which is applied to remove contaminant species, debris and sand that cannot be removed during any of the previous stages. It is only practical with smaller accessions.

Cleaning is pursued to a reasonable end point. If further cleaning will result in damage, or is too time consuming, then the final result may be a seed-lot containing some debris or empty seeds. Throughout, the cleaning method and the purity of the collection at storage is recorded. As will be appreciated, knowledge of the structure of fruit and seed heads is important to the entire cleaning procedure (see Dickie and Stuppy, 2003 – Chapter 15).

During the cleaning process, sieves and aspirators are kept clean to prevent cross contamination of collections. Similarly, working surfaces and floors are kept clean in case of accidental spillage of samples. Nearly all plant debris and waste material from the MSB cleaning process is disposed of in sealed biological waste bins. These are taken off site for very high temperature controlled incineration. This, added to a contained working environment and high levels of cleanliness, substantially reduces the risk of introducing pests and diseases from overseas.

Finally, where Access and Benefit-Sharing Agreements (ABSAs) permit, insects found in accessions are preserved in alcohol and forwarded to entomologists for identification, and certain plant debris is forwarded to RBG Kew's biochemists for analysis. New information about the plant's chemistry and insect species that live with the plant add scientific value to the collection.

### 3. X-ray Analysis or Cut-test

X-ray analysis can be an invaluable tool in determining the status of seed samples before, during or after seed cleaning. It is a means of estimating the proportion of empty, poorly developed or insect-infested seeds present in a sub-sample of the main collection. Where the X-ray analysis reveals an easily removable fraction of empty seeds, the collection is recleaned. Sub-samples of MSB collections are routinely tested after cleaning. X-ray analysis will often show the internal structure of a seed, including the size and position of the embryo.

However, unless contrast agents such as barium chloride are used (see Gosling, 2003 – Chapter 24), simple X-ray analysis *cannot* determine if a seed is alive or dead, merely that it looks potentially competent. Compared to a cut-test where the seed is dissected and examined under a microscope or hand lens, X-ray analysis is non-destructive, although some genetic damage is possible. In some instances, such as the analysis of the contents of complex, multiple-seeded fruits (e.g., those of *Beta vulgaris* L.), a cut-test is the only option.

The X-ray machine used for examining MSB seed collections comprises a high definition, water-cooled X-ray tube inside a radiation-proof cabinet (for product details, see Linington *et al.*, 1995). The higher the voltages applied to the X-ray tube, the shorter the wavelength of the X-ray beam and the greater its penetration of the denser parts of the seed and the contrast of the image on the sheets of special X-ray film. The greater the product of exposure time and amperage, the denser the image. The work is carried out within a dark room and the radiographs are developed by standard photographic techniques. They are then stored for future reference. The relatively safe nature of seed X-ray analysis is indicated by the fact that to obtain a satisfactory image of hard-coated *Acacia* seeds, a voltage of up to 20 kV may be used, while a human chest X-ray requires around 115 kV. Comparison of conditions required for obtaining reasonable radiograph images of seed from a variety of species is presented by Linington *et al.* (1995).

A large number of sub-samples can be X-rayed in a short period of time (see Section on ‘Staffing and Throughputs’) by one or two people, which makes the process relatively cost-effective in the UK. Apart from the dark room, the main capital expenditure has been the X-ray machine (£12,950 in 1990, perhaps – about £19,000 in US \$28,000 on 2002 values). A scintillation counter is used regularly to check the machine for any signs of radiation leakage while the machine is activated. X-ray film and developing chemicals are relatively inexpensive, if used economically, and are easy to obtain in the UK. An ordinary library microfiche reader is used to view the completed radiographs. An alternative would be to examine the radiographs with an illuminated box and a magnifier.

A fairly small sub-sample of seeds (approximately 50) can be analysed to give an acceptable estimate of the quality of the whole collection. The X-rayed sample is not returned to the batch; it is discarded, used for display or, occasionally and in the case of very small accessions, used for a germination test.

#### 4. Seed Quantity Determination

Several methods are used to estimate the number of seeds within accessions. The determination and method is recorded on the Seed Bank Database (SBD), which, where appropriate, calculates the final seed quantity. If fruits are to be stored and therefore counted, then the number of seeds per fruit must be recorded. The method used to estimate quantity depends on a number of factors:

- 1) For most collections, a 4 or a 7 decimal place balance (dependent upon seed size) is used to weigh five samples of 50 seeds or fruits. The remainder of the collection is then weighed. The upper 95% confidence limit for the weight of a 50 seed or fruit sample is calculated, which provides a general underestimate of quantity when divided into the total collection weight multiplied by fifty. Underestimating the seed quantity ensures that the collection will not be exhausted earlier than expected.
- 2) If seeds are exceptionally small (e.g., those of *Orchidaceae* and *Orobanchaceae*), a sample of 250 seeds is weighed, followed by the entire collection, and an estimate of the quantity is made accordingly.
- 3) If there are only a few seeds/fruits (less than about 300), they are counted individually.
- 4) A collection may be counted entirely by a seed counting machine if it is clean and has suitably sized seeds/fruits. However, this method is too slow for very large collections.

#### 5. Drying, Packaging and Cold Storage

Incoming seed accessions are unpacked, as detailed previously, and placed immediately into the first of two seed drying rooms for at least one week. This room is maintained at 15% relative humidity (RH) and 15°C, an internationally-accepted regime for drying collections. Bags are placed loosely in slatted plastic crates to facilitate drying. Collections that are tightly packed within bags or that are damp upon arrival may be spread in a thin layer within the crates to enable them to dry more evenly. After cleaning and quantity determination is completed, the accessions are moved to a further drying room. This room is currently maintained at 11% RH and 18°C, though RH conditions are likely to be adjusted upwards soon in the light of recent research (see discussion in Probert 2003 – Chapter 19). Seed-lots remain within the room for one month by which time they should have reached equilibrium with the air. Before being packaged ready for storage, their moisture status is checked by means of a hygrometer (see Probert *et al.*, 2003 – Chapter 20). A variety of containers are used in the MSB (see Manger *et al.*, 2003 – Chapter 34, for a full description). The cold storage at -20°C is described by Linington (2003 – Chapter 33).

## 6. Security Duplication

Up to half of each collection will already have been deposited in the country of origin. If suitable storage facilities do not exist in these countries, entire collections (fully processed and foil bagged) are held in the MSB at  $-20^{\circ}\text{C}$  until the country of origin requests repatriation of the half that it will store. Where the ABSA permits, a sample of 150 seeds is removed from the main collection (when size is  $>1,150$  seeds) and sent to the Scottish Agricultural Science Agency facility near Edinburgh in Scotland. Here, samples are stored in small bottles placed inside sealed, plastic boxes containing silica gel and held at  $-20^{\circ}\text{C}$  within freezers. These samples act as an added insurance against catastrophic loss within the MSB and could be used to regenerate collections.

## 7. Germination Monitoring

A germination test is ultimately the best test of viability, but dormancy (see Box 17.2) often poses problems. The viability of MSB seed-lots is assessed about one month after they are placed into the  $-20^{\circ}\text{C}$  cold store. Testing them at that time checks that seeds of a given species are able to withstand the banking process. Germination is subsequently re-tested at approximately 10-year intervals. The basic test procedure is very simple:

- 1) A seed container is removed from the cold room and allowed to warm up for one day in the adjacent drying room. A suitable quantity of seeds is then removed. The quantity is the product of the number of dormancy-breaking treatments at a given stage of testing (see Box 17.3) and the treatment sample size. The number of seeds in each treatment sample is usually 20 or 50 seeds, dependent upon the total number of seeds in the seed-lot. For very small collections, as few as 10 seeds may be used, although seed-lots with less than 500 seeds are usually left untested. Very small germination tests do not yield data capable of statistical analysis but do give an indication of success of dormancy-breaking treatments and general level of viability.
- 2) The seeds are sown onto  $10\text{ g l}^{-1}$  water agar (see Box 17.1).
- 3) The test is placed at an appropriate temperature in an illuminated incubator (8 h fluorescent light/16 h dark).
- 4) Germination is recorded (usually a 1–2 mm protrusion of radicle) at weekly intervals and the germinated seeds removed and discarded. Although radicle emergence is a relatively lax measure of germination, it appears possible to relate it to other measures such as radicle-plus-shoot growth and viability as determined by vital staining (see Ellis and Roberts, 1981). Tests are usually examined in a clean air cabinet to minimise the risks of inhalation of fungal spores produced by any mould growing on the seed.

**Box 17.1 Use of agar as a germination medium**

As a general rule, plain distilled water agar at 10 g l<sup>-1</sup> is the preferred substrate for germination testing of the MSB seed collections. There are several distinct advantages:

1. Imbibition injury, which can occur when very dry seeds absorb water too quickly, is minimised when seeds are sown on agar.
2. Agar is very easy to prepare. Preparation simply involves dissolving the agar powder in boiling water and pouring it into Petri-dishes before it starts to solidify. Dormancy-breaking chemicals including hormones such as gibberellins can be readily incorporated.
3. In the UK at least, agar is relatively cheap compared to substrates such as filter paper.
4. Due to slow evaporation of moisture from the medium, agar is a relatively low maintenance substrate for germination compared to filter paper or sand.
5. Because water agar contains little in the way of nutrients to support microbial growth, it is a relatively inert medium that does not require strict sterile technique during handling.
6. When used in a glass or plastic Petri-dish, it is possible to see pale radicle emergence clearly against a dark background.
7. Because the radicles grow down into the agar, it is possible to cut out agar plugs holding individual seedlings and to successfully transplant them into soil or compost. This is not so easy with seedlings grown on filter paper.

- 5) When germination has stopped (no germination occurs during two weeks following four weeks of testing), the test is terminated and the remaining seeds are evaluated by visual inspection and a cut-test to ascertain whether they are full, empty or mouldy. Excessively mouldy, yet filled, seeds indicate loss in viability, particularly if very soft. For wild species it is not unusual for each test to take many weeks or even months. The germination pass level (Regeneration Standard) for the MSB collections is 75% ( $P < 0.05$ , one-tailed significance test) after empty and damaged seeds are taken into account. Failures may be regenerated (see below), discarded or kept, dependent upon the rarity of the material, the level of germination and the potential ease of regeneration. Re-collection may be considered for some material, if possible.

The particular germination conditions chosen for a given species will usually be determined by family-based test schemes (see Box 17.3), developed from data accumulated over many years about collections from the Kew Seed Bank and its successor, the MSB. These are enhanced by information from literature surveys. As the test schemes are developed using data from a wide range of genera they can often be successfully applied across the whole family.

**Box 17.2 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 (see also Baskin and Baskin, 2003 – Chapter 28). 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, disposal. The development of practical treatments for the removal of seed dormancy is thus one of the most important roles for seed conservationists.

**Examples of the principal types of seed dormancy encountered by the MSBP.**

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

**8. Drying and Mounting of Voucher Specimens and their Verification**

When batches of seed arrive, the accompanying herbarium vouchers are unpacked and checked. The vouchers must be dry, correctly labelled and free from pests. Any specimens that are not completely dry are separated from the remainder of the batch. The wet specimens are either placed inside fresh drying papers in herbarium presses in the drying room or, if very wet, placed in a 40°C oven. All specimens are regularly checked. Once dry, the vouchers are packed in plastic bags and placed at -20°C for one week. This kills any insects present. After freezing, each specimen has a herbarium label printed from the SBD and placed with the plant material. Batches of vouchers are logged on the SBD and sent to the Kew Herbarium for mounting and identification. This identification is then entered on the SBD. Any specimens that are insufficient for identification are noted and a fresh specimen is then grown in the WTMB glasshouse. After the voucher is harvested in the glasshouse at the required stages of flowering and fruiting, the subsequent procedure is as described above.

**Box 17.3 Family-based germination schemes**

The stepwise approach to germination testing of the MSB collections has enabled the development of family-based 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 treatment. The following are examples of three family germination schemes (tests are terminated when a successful result is achieved):

***Asteraceae (Compositae)\****

- I Constant temperatures of 10, 15 and 25°C
- II Alternating temperatures of 25/10 and 35/20°C
- III 1 week at best temperature, sterilise (10% domestic bleach, usually for 5 minutes), excise embryo and incubate at 15°C
- IV Cold stratification of seeds on agar for 8 weeks at 5°C, then best germination temperature
- V Apply chemicals – potassium nitrate (0.1 g l<sup>-1</sup>), Gibberellic Acid GA3 (0.25 g l<sup>-1</sup>) using best germination temperature
- VI Tetrazolium (TZ) test

\* See Lington *et al.* (1996)

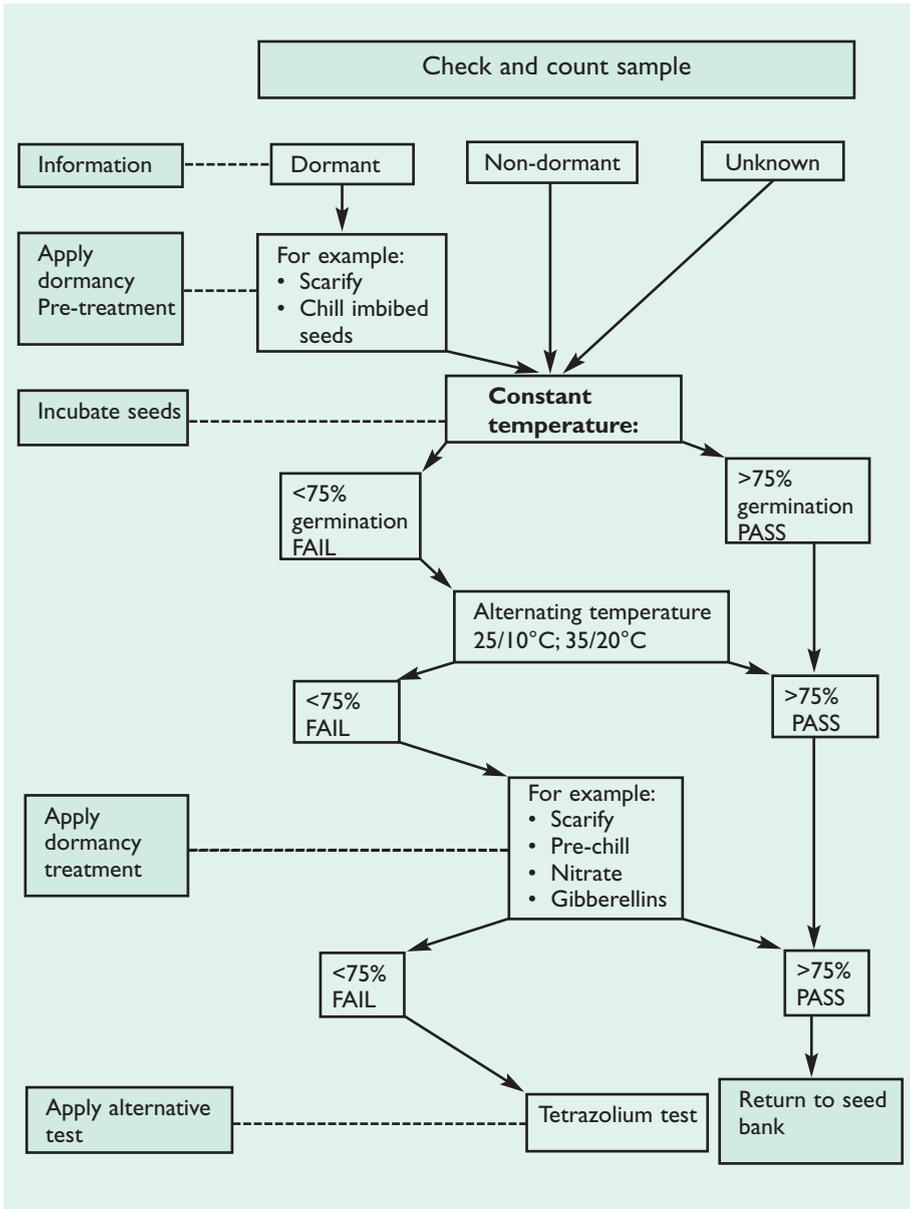
***Fabaceae (Leguminosae)***

- I Scarify by scalpel chipping or file, then constant temperature of 15 and 25°C
- II 'Condition' by holding seeds over water in a sealed box for 2–4 days at 20°C, then germinate at best temperature
- III Tetrazolium (TZ) test

***Liliaceae (sensu lato)***

- I Constant temperatures of 15 and 25°C
- II Cold stratification for 8 weeks at 5°C, then best germination temperature
- III Surgically excise endosperm to expose embryo, then best germination temperature
- IV Tetrazolium (TZ) test

If a test scheme is not available for a particular family, the seed-lot is examined carefully and a range of conditions is applied that are considered suitable (see Figure 17.1). Failure of tests leads to more detailed research and TZ staining (see Box 17.4).



**Figure 17.1** Typical MSB 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. This enables end users to 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.

**Box 17.4 The approach to tetrazolium testing of MSB seed-lots**

The International Seed Testing Association (ISTA) recommendations for tetrazolium (TZ) testing (Anon, 1999a & b) are an excellent guide to the general methodology of TZ testing, and for a range of species of agricultural and horticultural importance, specific guidelines are provided on both the method and evaluation of tests.

TZ tests are routinely employed to distinguish between dead and dormant seeds when germination tests of MSB collections fail to reach a pass (i.e., < 75% viable seeds, with  $P < 0.05$ , one-tailed significance test). In view of the extreme diversity of species encountered in the MSB collections, and for which ISTA guidelines are mostly unavailable, a simplified approach has been developed that gives reliable results in the majority of cases. The essential elements are outlined below. See Anon (1999b) for other details.

1. Sample size is as for germination
2. Pre-moistening. Seeds are imbibed on filter paper, wetted with distilled water, for 24–48 h at 20–25°C in order to rapidly and conveniently soften the tissues for the next step. Hard, impermeable seeds are scarified before they are transferred to wetted filter paper. A more gentle approach is to imbibe seeds in an atmosphere of 100% RH for 24 h before placing on agar. This is more suitable for accessions that are suspected of being of low viability.
3. Exposure of vital tissues. This is probably the most important step and requires an understanding of the structure and anatomy of the seed to be tested (see Dickie and Stuppy, 2003 – Chapter 15). Thus when dealing with unfamiliar seeds, a few are first dissected and details annotated so that the nature of the internal tissues can be established. The most important feature is the location of the embryo within the surrounding tissues. When this is established, the seeds to be tested are carefully cut or dissected to expose the vital tissue.
4. Incubation. During the incubation of TZ tests, it is important that the seeds are fully immersed in the TZ solution and that the test is kept in the dark to prevent photo-conversion of the solution. Tests are usually incubated at 30°C for 24 h. Higher or lower temperatures respectively speed up or slow down the rate of staining.
5. Evaluation. Prior to evaluation, the TZ solution is discarded and the seeds transferred to wetted filter paper. For a range of species, the ISTA guidelines indicate the relationship between precise patterns of staining and seed viability. These may be a useful guide to other related species. However, SCD staff have found that a simple classification, based on overall staining, works in most cases. Essentially, the aim is to distinguish between seeds that are viable, seeds that are dead and seeds that are dying. Accordingly, the seed tissues are inspected under a dissecting microscope (additional tissue exposure may be necessary) and then the sample is divided into three simple staining categories:

Vital tissues more or less uniformly well stained red	Viable
Vital tissues patchy or weakly stained pink	Dying
Vital tissues more or less completely unstained	Dead

For analysis of results, see Box 17.5. To assist the judgement of the above categories, the magnification of the microscope is adjusted so that all seeds in the sample can be viewed simultaneously. This helps to remove some of the inevitable subjectivity in this method.

**Box 17.5 Analysis of tetrazolium results**

Based on the evaluation described in Box 17.4, the staining patterns are evaluated and an estimate made of percentage viability of the collection. One of the main concerns is to ascertain whether the viability of the sample is significantly higher than the proportion of seeds that have germinated. In other words, whether there is a proportion of dormant individuals that have not germinated. If this is the case, additional tests may need to be carried out to overcome the dormancy problem. To check this, a proportions test is applied using Pearson's chi-squared statistic with Yates' continuity correction to compare the proportion of individuals that germinated in the germination test with the proportion indicated as viable in the TZ test. If the results are not significantly different ( $P > 0.05$ ) the germination result is accepted as a true indication of viability.

**9. Distribution of Sub-samples to Users**

A seed list is regularly produced. To date, this has been a booklet. In future, it is anticipated that a list will be made available on the RBG Kew website with access controlled by registration. The list comprises collections for which distribution is permitted. Included are collections subject to those ABSAs that permit supply under certain terms and conditions. Listed collections have been identified (usually from a herbarium specimen or living material), have more than 1,050 filled seeds and have successfully completed a germination test in the last 10 years. Up to 25 samples, each of 50 seeds, are available free of charge to organisations carrying out *bona fide* non-commercial research. Where approved by relevant statutory agencies, they are also made available for (re-)introduction programmes. Seeds are not made available to private individuals. All organisations requesting material must first sign a legally binding Material Supply Agreement (MSA) before samples are supplied. This states that the material must not be used for commercial purposes or passed to a third party. Where benefits such as research results or publications arise, these must be shared with RBG Kew so that they can be forwarded to the country from which the sample originated. A user wishing to commercialise the material, its progeny or derivatives would need to conclude a separate agreement with the appropriate stakeholders. Users are also asked to check import arrangements (with respect to plant health), and are cautioned with respect to the weed potential of alien species. Finally, users are also asked to detail the intended use of the seeds.

Seed orders are processed on the SBD. It automatically deducts numbers of seed used from the seed quantity field and generates the required paperwork. Seeds are counted out into glassine paper envelopes and then packed in paper envelopes labelled with the serial number and species. The batch of samples for each customer is sealed in a laminated foil bag. This whole operation is carried out in the dry room, preserving the low moisture content of the seeds. The customer additionally receives instructions for germination and a copy of the MSA.

Uses of the seed samples around the world have included studies on biological control, desert adaptability potential, evolution, gums and resins, nectar sources, nitrogen fixation, ozone sensitivity, pasture improvement and ringworm treatment. A number of the samples are distributed for more general university and public education. One example of a collection being used for re-introduction studies is *Cerastium brachypetalum* Pers. where seeds were made available to determine the best mitigation measures for re-establishment after destruction of its habitat by the UK-France Channel Tunnel Rail Link.

## 10. Glasshouse Work

The WTMB has a single large glasshouse divided into four individually computer-controlled zones. Additionally, several cooler glasshouses are available nearby. Plants are grown for several reasons:

- 1) *To grow plant material for herbarium voucher specimen preparation.*  
This may be for a collection where no voucher was supplied from the wild, where the wild collected voucher was insufficient for identification or to check identification in the case of a seed collection that appears to include more than one species. Sometimes, flowers are produced after prolonged cultivation, e.g., seed stocks of *Puya* cf. *tovariana* were grown out in 1994, but it was not until 2001 that the plants finally flowered.
- 2) *For the multiplication of plants to produce large seed harvests.* This mainly applies to species in the MSB that are poorly represented and that have very low seed numbers. Usually these species will be rare UK natives, but they may also be from overseas. An example is *Cylindrophyllum hallii* L.Bolus from South Africa. This plant is critically endangered in the wild. Plants are being cultivated in order to increase seed numbers. The plant material will be distributed to other *ex situ* conservation organisations and will be used to develop a propagation protocol. Other collections are also multiplied for re-introduction into the wild. To date these have been mainly UK native species such as *Damasonium alisma* Mill. (Starfruit), *Corrigiola littoralis* L. (Strapwort) and *Apium repens* (Jacq.) Lag. (Creeping Marshwort). This work has primarily been in support of English Nature's Species Recovery Programme. The MSB Project has also been involved in the successful propagation of plants of *Silene tomentosa* Otth in DC. from Gibraltar, a species thought to be extinct in the wild. Plants were grown from seeds stored in the MSB. Using the knowledge gained from this process, plants have now been successfully reintroduced back onto the Rock of Gibraltar.
- 3) *For regeneration of seed-lots where the viability has fallen below 75%.*

- 4) *For the provision of research and experimental material.* Plants may be grown for a variety of reasons. For example, in a recent study of seed dormancy problems in tropical grasses, MSB collections of *Eragrostis pilosa* (L.) P.Beauv. and *Eragrostis cylindriflora* Hochst. were regenerated to produce sufficient quantities of seeds for experimental purposes. The resultant, highly dormant, seed-lots are being used to develop novel dormancy-breaking treatments based on fire simulation.
- 5) *To provide living material for public display purposes.* Many examples of species native to the United Kingdom have been propagated. These are displayed in eight raised beds (parterres) outside the WTMB. Each parterre represents a different habitat found in the UK. Additionally, the WTMB has a raised bed inside the public interpretation and viewing area. This contains dryland species represented within the MSB. As well as providing plant material, this process also provides horticultural knowledge of species that may not have been grown in cultivation previously.

## 11. Staffing and Throughputs

The staff who carry out the processing and maintenance work on the MSB collections are the Curation Section of RBG Kew's Seed Conservation Department. Batches are processed from arrival to germination by one of two teams, each team comprising of a supervisor and three assistants. A Processing Manager is in charge of co-ordinating and directing the teams. A third team will be recruited when seed intake warrants it. Estimated throughput times for collections processed into the MSB are shown in Table 17.1.

With an estimated total staff time of 11,411 h per annum available for the work activities included in Table 17.1, a maximum throughput of 3,118 collections per annum should, in theory, be achievable.

**Table 17.1 Estimated throughput times for collections processed into the MSB**

Process	Staff time per collection (h)
Accessioning (entering collecting data)	0.22
Cleaning	2.32
X-ray analysis and counting	0.25
Banking	0.17
Germination	0.70
Total	3.66

One unusual aspect of working in the Wellcome Trust Millennium Building is that staff are in the public gaze from visitors to the building's interpretation area (the Orange Room). This has not caused undue difficulties and has helped the public understand the work and purpose of seed banks and, more widely, that of plant conservation.

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