

This protocol is used by the MSB to compare the seed longevity of different species held in the seed bank.

The method aims to generate a single seed survival curve, using a carefully controlled ageing environment. Seeds are first rehydrated at 47% RH, 20°C and then aged at 60% RH, 45°C, using Lithium Chloride (LiCl) solutions to provide the desired relative humidity (RH) environments.

Relatively few seeds are used, so the method is ideal for conservation collections.

The controlled ageing test generates a measure of the longevity of a collection that can be compared with the longevity of 'marker' species under the same conditions. Therefore, whilst the method does not allow accurate prediction of seed longevity for the test species, comparison across species and with markers enables the ranking of species into longevity categories. The same method can also be used to investigate the effects of factors such as maturity or post-harvest handling on seed quality.

Seed requirements:

- 600 seeds per test: 10 x 50 for germination testing; 2 x 50 for moisture content determination.
- For comparative longevity testing of conservation collections only large collections from which 600 seeds can be spared should be used. They should also have high (>85%) known viability and no serious constraints to germination testing.

Right: Electrical enclosure box containing seed samples held above non-saturated LiCl solution:

For rehydration add 370g LiCl to 1 litre distilled water (47% RH)

For ageing add 300g LiCl to 1 litre distilled water (60% RH)

Preparation

Take collections from cold storage and allow them to warm up to room temperature before opening containers to avoid condensation forming.

Count seeds into 12 samples of 50. Place them in open glass vials or dishes of suitable size, in a monolayer.

If the seeds require 'chipping' or 'dehusking' for germination, then perform these treatments prior to rehydration.



Monolayer of seeds ready for rehydration

Rehydration: 47% RH, 20°C

Place glass vials / dishes in box at 47% RH and 20°C. These conditions minimise any change in seed moisture content when the samples are transferred to the ageing conditions. As the seeds warm up from 20 to 45°C the equilibrium relative humidity (eRH), or water activity, of the seeds will adjust to 60% eRH.

The rehydration period is usually two weeks. However, this is dependent on seed size, so larger seeds may require more time. Check seed eRH to ensure that equilibrium has been attained using a suitable hygrometer such as the Rotronic Hygropalm.

For 'small' seeds, an eRH reading will be inaccurate because the sample of 50 seeds will not fill the hygrometer sample chamber sufficiently. For such species, a 'dummy' collection, with a larger volume of seeds suitable for eRH measurement, is set up in the rehydration environment and the eRH of this collection is measured. Any species with suitably sized, permeable seeds could be used.

Once equilibrium is reached, take one sample of 50 seeds and measure seed moisture content (17 h at 103°C). Move the remaining samples to the ageing environment.



Ageing: 60% RH, 45°C

The open storage environment created inside the sealed box ensures that seed samples under test experience identical ageing conditions. The container is held in a fan assisted oven in the dark.

Withdraw samples at random on the following days:

Day: 1, 2, 5, 10, 20, 30, 50, 75, 100, 125.

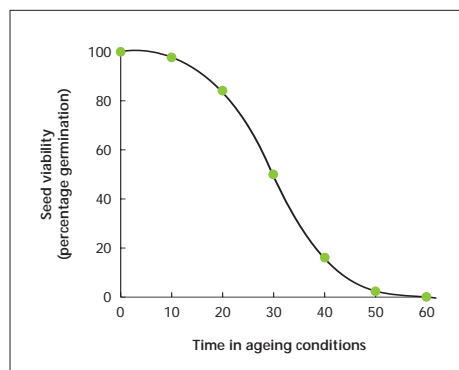
Sow each sample as a germination test, under appropriate germination conditions. Run each test for at least 42 days, and until there has been two weeks without any germination. Perform a 'cut test' at the end of each germination test, to confirm that any ungerminated seeds are dead and not otherwise incompetent. This is an important part of assessing the viability of the seeds. Note any abnormal seedlings but do not score as germinated.

Approximately mid-way through the ageing period, use one sample for a second moisture content determination to check the stability of the ageing conditions.

The sampling schedule shown above was designed for species of unknown longevity. For species expected to be short- or long-lived adjust the sampling schedule accordingly. Even for new species, provided that germination is rapid, there may be an opportunity during the test to widen the sampling intervals if the germination data indicates that the seeds are showing little sign of viability loss.

Analysis

The germination data is plotted as a seed survival curve, where seed viability is plotted against ageing period.



Typical seed survival curve

The time for viability to decline to 50% (P_{50}) or mean time to death (MTD) can be easily calculated from seed survival data. Seed survival curves are usually analysed by a weighted linear regression after converting the viability data to probit values (probit analysis). A value for sigma is calculated, which is the time for viability to fall by one probit. Sigma values are then used to rank the species, allowing comparisons to one another, and to the markers in the screen.

Practical tips:

- Once a month, check and adjust the eRH of the LiCl solutions, to take account of the desorption of water that occurs over time, as the box is opened to put in and take out samples, and through leakage. Measure a few mls of the solution directly in a hygrometer sample chamber, taking care not to contaminate the sensor head. Experience at the MSB has shown that in boxes at 60% RH and 45°C containing 1 litre of solution in constant use, the eRH falls by approximately 2% every month. Add approximately 40 ml H₂O to counter this. The boxes at 47% RH and 20°C need adjustment less often.
- Use glass vials and Petri dishes rather than plastic dishes to minimise problems of static electricity.
- Two 'marker' species with known viability constants used at the MSB are *Ranunculus sceleratus* and *Brassica napus*. The longevity of test species are compared with these.

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Equipment specifications:

- Seed containers (available from www.fisher.co.uk):
 - 2 ml clear Wheaton-style vial. Catalogue No: VGA-220-012C
 - 5 ml clear Wheaton-style vial. Catalogue No: VGA-220-121U
 - Glass Petri dishes 52 x 12 mm. Catalogue No: PDS-100-011U
- Ensto Electrical Enclosure Box 300 x 300 x 102 mm, conforming to IP67. www.ensto.com
ABS Base Product Code: OABP303010B; Clear lid Product Code: OPCT303003L
- LEEC KIF Compact Incubator, via Jencons-PLS. www.jencons.co.uk
- Rotronic HygroPalm AWVC-DIO sensor with Palm 3 display unit. www.rotronic.co.uk

