

Germination tests are used by the Millennium Seed Bank (MSB) for two reasons:

1. To monitor the viability of seed collections.
2. To develop protocols for turning seeds into plants.

Initial viability is tested once collections have been stored at  $-20^{\circ}\text{C}$  for at least 7 days, and ideally within 3 months of banking. Viability is then monitored at least every 10 years.

Germination testing is often the most reliable way of assessing viability. It is important to monitor viability as non-viable seeds may not be apparent at other stages of processing. Optimal germination conditions should allow all viable seeds to germinate using the simplest method possible. For more information on choosing appropriate germination conditions, see [Technical Information Sheet\\_13b](#).

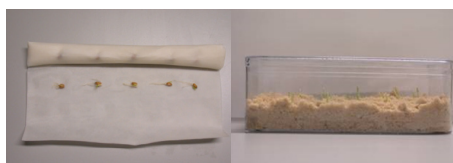
## Preparing for germination testing

### Sample size

It is important to know the total number of seeds available in the collection, including the portion expected to be empty or infested. This portion should be assessed after the cleaning process using an x-ray or cut test, see [Technical Information Sheet\\_14](#). You need to sow enough full seeds to allow proper analysis, without depleting an important collection (see table to the right).

### Germination medium

Choose a germination medium such as 1% agar, sand (particularly useful for large seeds) or germination paper. Agar is highly suitable as it is clear and so easy to see the seeds, retains moisture, and can be combined with chemical compounds such as gibberellic acid ( $\text{GA}_3$ ). If using a medium other than agar, take care not to add too much water (it should not pool on the sand / paper) or to let it dry out.



Above - Alternative substrates: germination paper (left) and sand (right).

### Germination containers

Choose a germination container (Petri dish, box, etc.) that allows light to reach the seeds, and which is large enough to contain all the seeds without overcrowding. Seal containers inside a plastic bag to prevent moisture loss and reduce contamination.

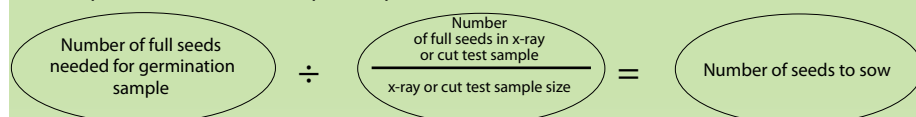
Collection size*	Test size*	Number of tests**
$\geq 1000$	25 - 50	4 - 10
$\geq 500$	25	2 - 4
$\geq 250$	10	2 - 4
$< 250$	No test	0

\* Excluding empty / infested portion (see over-sowing calculation below)

\*\* No more than 10% of the collection should be used

### Over-sowing

When germination testing collections with empty or infested seed, sow extra seed to compensate for the incompetent portion.



E.g. if 45 / 50 seeds full:  $50 \div (45 / 50) = \text{sow } 56 \text{ seeds.}$



### How to make 1 litre of 1% agar

- Weigh out 10g of agar powder into a large jug and mix to a smooth paste by adding 100ml of cold distilled water.
- Add 900ml of boiling water to the agar paste and stir well.
- Gently heat the solution, stirring continuously until boiling.
- Allow to cool to approximately  $50^{\circ}\text{C}$  before pouring.

1 litre of agar yields approximately 33 x 9cm Petri dishes (30ml per plate)

### To add gibberellic acid ( $\text{GA}_3$ ) to agar:

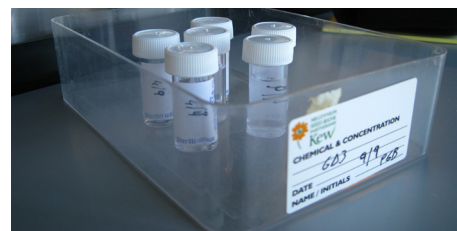
- Add 5ml of stock solution (see below) to 445ml of boiled agar (cooled to  $50^{\circ}\text{C}$ ) and pour plates as before.

### To prepare stock solutions of $\text{GA}_3$ (250mg/l)

- Add 4.5g of  $\text{GA}_3$  to 200ml\* cool deionised water.
- Adjust pH to 6.5 using sodium hydroxide / hydrochloric acid.
- Filter the solutions using a sterile Nalgene filter and syringe in a laminar flow cabinet.

The stock can be stored for 3 months at  $5^{\circ}\text{C}$  in the dark.

\* To ensure final volume is not  $>200\text{ml}$ , start with 180ml and adjust after setting pH.



Above -  $\text{GA}_3$  stock solutions at the MSB

## Setting germination tests

Prepare a germination test sheet for each collection (see example test sheet on back page).

Label each germination container and test sheet clearly, including;

- date started
- collection number
- species
- number of seeds sown
- germination temperature
- any additional treatments

**Sowing** the seeds evenly in a grid can reduce the spread of mould and allows easy inspection.

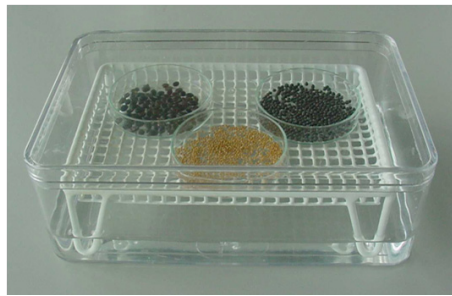
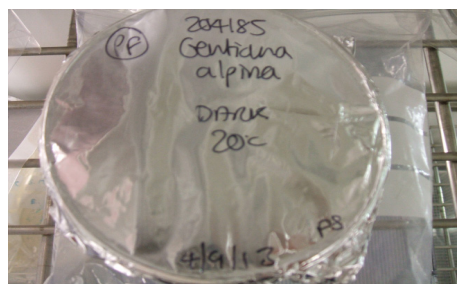


## Incubators

Suitable incubators for germination have low energy, cool white fluorescent tubes. Incandescent lamps should be avoided as they produce too much far red light and heat. Photoperiod should coincide with the thermoperiod, with a cycle of 8/16 or 12/12 (light / dark) hours.



Wrap **dark germination** tests in aluminium foil as below. Check for germination in a dark room under green light.

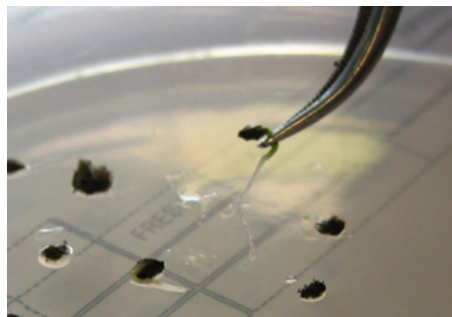


**Rehydration** (above) can reduce risk of imbibition damage, especially where a soaking treatment is applied (e.g. sanitisation). Suspend dry seed over water in a sealed container for 24 hours before sowing.

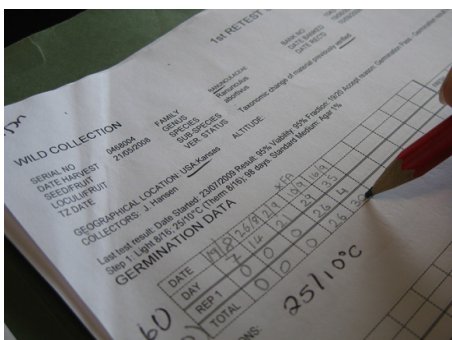
## Sanitisation

Seeds can be sanitised by soaking in a 0.5% sodium hypochlorite (NaOCl) solution, containing a 1% surfactant (Tween 20) for 10 minutes and then rinsing under running water for 1 minute.

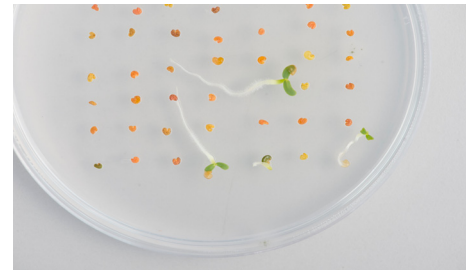
## Germination test monitoring Scoring



Score germination weekly, removing seedlings when the radicle is at least 2mm long (for very small seeds, shorter radicles are acceptable). Record the date, days after sowing, and number germinated on the test sheet. Record abnormal seedlings (e.g. those with cotyledon growth only) separately.



Above - Record weekly scoring data on the germination test sheet.



Place seeds on fresh substrate as necessary (e.g. to control fungal contamination or remove inhibitors leached from the seed coat) noting this on the test sheet each time. Chemical additives (such as GA<sub>3</sub>) should be renewed every four weeks, as these degrade over time.

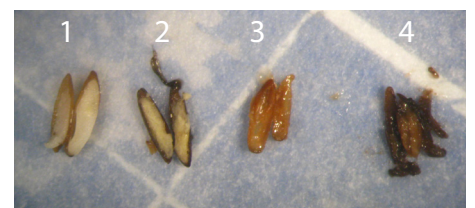
## Test duration

Continue tests until germination stops or all seeds have germinated. If there is no germination after 42 days, or germination has stopped for more than four weeks, decide whether to continue the test (e.g. slow germination expected), apply a dormancy breaking treatment (see [Technical Information Sheet\\_13b](#)) or end the test. This decision may depend on the family, embryo size, seed health, etc. Seeds of some wild species can take years to germinate!

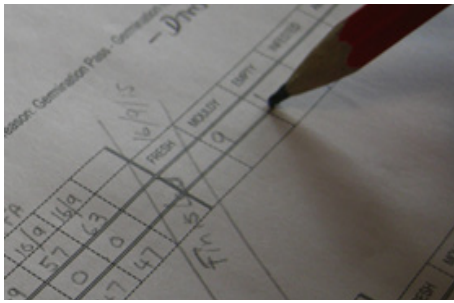
## Cut testing



It is important to dissect any seeds that have not germinated at the end of the test. This will allow proper evaluation and interpretation of the result. Paying special attention to the embryo, record each seed as fresh, mouldy, empty, insect-infested or abnormal. Enter the results on the germination test sheet.



Above - Cut test examples of fresh (1), mouldy (2), empty (3) and infested (4) *Najas flexilis* seeds.



Above - Record cut test data on the germination test sheet.

## Germination test evaluation

### Calculating percentage germination and viability

Empty and insect-infested seeds are excluded from the calculations as they could never germinate. This fraction (unlike viable / non-viable seeds) does not change over time and so does not need monitoring in a germination test. Abnormal seedlings are recorded as fresh but not germinated. To calculate percentage germination and viability use the following equations:

$$\text{Germination (\%)} = G / X * 100$$

$$\text{Viability (\%)} = (G+F+A) / X * 100$$

where:

G = number of seed germinated

X = number of seed sown (excluding empty and infested)

F = number of fresh seed

A = number of abnormal seedlings

### Interpreting germination results

High viability collections should achieve a germination result above 85%, meeting the international regeneration standard (FAO, 2014). However, statistical analysis can also be used to make decisions on collections of any viability.

The most successful test (achieving highest germination with the simplest method) can then be 'accepted' and those same conditions used for any future testing.

### Cut test reliability

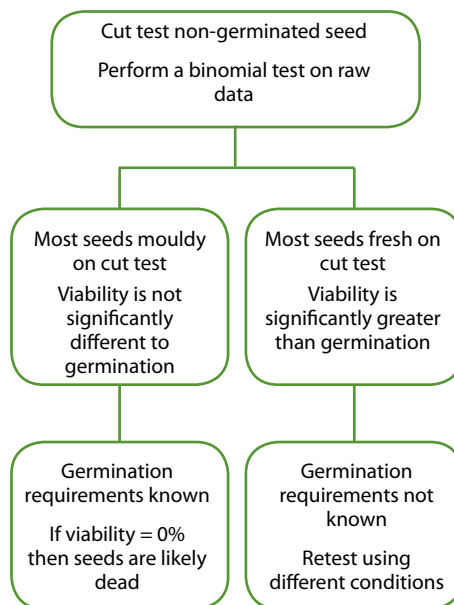
It is important to remember that viability calculated from a cut test is only an estimate. Assessing viability across all tests and including controls should increase the reliability of tests assessed in this way.

If a large proportion of seeds do not germinate but appear 'fresh' at the end of a test, it is likely that the test conditions used were not optimum for that collection or that the seeds were dormant.

Conversely, if remaining seeds are 'mouldy' on dissection, it can be assumed that all viable seeds have germinated and so germination cannot be improved. Low germination in this case could suggest a low viability collection.

### Statistical analysis for germination tests

To assess whether germination is significantly less than viability, a two-sample, one-sided binomial test can be used. If there is no significant difference between viability and germination (i.e. most or all viable seeds have germinated) germination conditions are considered suitable.



As with other statistical tests, sample size is important. Tests of less than 10 seeds are too small for meaningful statistical analysis.

### Recording data and databases

If you want to analyse or share your germination data, it is important that you record all raw data in an appropriate format. Use a data management system with recognised seed bank data standards such as BRAHMS software (BRAHMS, 2015), which is capable of exporting data in a standard format.

### Monitoring collections over time

Management decisions (e.g. to collect again or regenerate) should be implemented if / when collection quality drops to <85% of initial viability. To begin with, viability should be monitored at least every 10 years (MSBP Seed Conservation Standards, 2015).

Test intervals should be reduced (e.g. from 10 to 5 years) when the first significant decline in viability is detected. Where possible, duplicate short-lived collections to cryo-storage. Where no decline is shown after at least three retests, intervals can be extended (e.g. from 10 to 20 years). This can help to reduce staff costs and conserve small seed collections.

Analyse retest data using a Z-test (Ellis et al., 1985) or by probit analysis where possible. See [Technical Information Sheet\\_01](#) in this series for further details on Probit analysis.



### Health and safety

- Scoring of tests should be carried out in a dust containment hood (above) to prevent inhalation of fungal and bacterial spores.
- Take care when handling chemicals; safety glasses and disposable gloves should be worn.
- Dispose of used scalpel blades in a sharps container.
- Clean all surfaces after use with disinfectant.
- Wash hands with biocide soap after working with seeds.
- Treat all seeds as though poisonous.
- Wear a lab coat.

### Further reading

Refer to [Technical Information Sheet\\_13b](#) for references.

### TEST SHEET

#### COLLECTION

SERIAL NO	FAMILY	BANK NO	SEED QTY	ADJ SEED QTY
DATE HARVESTED	GENUS	DATE BANKED	NO. X-RAYED	
SEED PER FRUIT	SPECIES	DATE RECEIVED	NO. EMPTY	
TZ RESULT	SUB-SPECIES		NO. INFESTED	
TZ DATE	VER. STATUS		NO. PART-FILLED	

GEOGRAPHICAL LOCATION: \_\_\_\_\_ ALTITUDE: \_\_\_\_\_

#### GERMINATION DATA

X	DATE						FRESH	MOULDY	EMPTY	INFESTED	ABNORMAL	DATE	STARTED
	DAY												
	REP 1												
	TOTAL										%GERM		

CONDITIONS:

NOTES:

Above - Example of a blank germination test sheet. Include all relevant collection information on the test sheet including taxonomy, collection data (location, date) and processing data (collection size, proportion of empty and infested seed).

#### Equipment specifications

Description	Model/Product	Supplier
Plastic germination containers	<ul style="list-style-type: none"> <li>Disposable Petri dishes SLS Select 90mm triple vent (SLS2002)</li> <li>Box clear PS 174x115x60mm (6145)</li> </ul>	Scientific Laboratory Supplies <a href="http://www.scientific-labs.com">www.scientific-labs.com</a>
Germination substrates	<ul style="list-style-type: none"> <li>Agar powder Fisher Bioreagents (BPE2641-1)</li> <li>White seed germination blotter 2" circle (WDB2), 3.25" circle (WDB3.25) and 9x6" rectangle (custom size)</li> <li>Sand (meets ISTA guidelines)</li> </ul>	Fisher Scientific UK Ltd <a href="http://www.fisher.co.uk">www.fisher.co.uk</a> Anchor Paper Company Seed Solutions <a href="http://www.anchorpaper.com/index.php/seed-solutions">www.anchorpaper.com/index.php/seed-solutions</a>
Chemical additives to germination substrates	<ul style="list-style-type: none"> <li>Gibberellic acid BioReagent (G7645)</li> <li>Potassium nitrate 99.5+% for analysis Certified AR (P/6120/53)</li> </ul>	Sigma-Aldrich Company Ltd. <a href="http://www.sigmaaldrich.com">www.sigmaaldrich.com</a> Fisher Scientific UK Ltd <a href="http://www.fisher.co.uk">www.fisher.co.uk</a>
Germination incubators with appropriate lighting and temperature regimes	<ul style="list-style-type: none"> <li>LMS cooled incubators</li> <li>280 cyclic with timed lights, autodefrost</li> <li>Series 1A available in various sizes</li> </ul>	LMS Ltd <a href="http://www.lms.ltd.uk">www.lms.ltd.uk</a> or <a href="http://www.ecomcat.com">www.ecomcat.com</a>
Containment hood for scoring germination tests	Powder handling workstation with HEPA filtration (XIT Plus 804)	Bigneat Ltd <a href="http://www.bigneat.com">www.bigneat.com</a>
Dissecting microscopes	<ul style="list-style-type: none"> <li>Nikon binocular stereo zoom microscope (SMZ445) with Photonic cold lightsource with gooseneck (PL1000)</li> <li>Lynx stereo microscope</li> </ul>	Jencons Scientific Limited <a href="http://www.ecomcat.com">www.ecomcat.com</a> Vision Engineering <a href="http://www.visioneng.com">www.visioneng.com</a>
Laboratory coat and disposable gloves		Locally available
Permanent waterproof marker pens	Stabilo Write-4-all	Locally available
Dissection kits	Kit no. 2 (AGT5242)	Agar Scientific Ltd <a href="http://www.agarscientific.com">www.agarscientific.com</a>
Disinfectant (for bench and equipment)	<ul style="list-style-type: none"> <li>Distel/Trigene Advance Disinfectant 5L Green (1% solution made up)</li> <li>Ethanol 99.8+% (GLC) 0.7897g/ml absolute duty free (70% solution made up)</li> <li>Tween 20 or Polysorbate 20 nonionic detergent</li> </ul>	Scientific Laboratory Supplies <a href="http://www.scientific-labs.com">www.scientific-labs.com</a> Sigma-Aldrich Company Ltd. <a href="http://www.sigmaaldrich.com">www.sigmaaldrich.com</a>
Disposable paper towel		Locally available
Aluminium foil for dark germination		Locally available
Surface sterilising solution	Sodium hypochlorite and Tween in de-ionised water	

Please note that the above equipment is used by the Millennium Seed Bank Partnership and has been chosen carefully using our many years' experience. The list of suppliers is for guidance only and does not represent an endorsement by the Royal Botanic Gardens, Kew. The manufacturer's instructions must be followed when using any of the equipment referred to in this Information Sheet.