
# ENSCONET
# Germination Recommendations
# UPDATED

Overall editor:
Royal Botanic Gardens, Kew

Version: 28 October 2009

ENSCONET
European
Native Seed
Conservation
Network

Citation: ENSCONET (2009) ENSCONET Germination Recommendations - Updated

This update was based on discussions at the

"ENSCONET Germination workshop"

held at the

National Botanic Garden of Belgium
Domein van Bouchout
Nieuwelaan 38
1860 Meise
Belgium

15-17 September 2009

*For more detailed information on germination please consult the ENSCONET Curation Protocols and Recommendations (ENSCONET 2009; www.ensconet.eu/download).*


ENSCONET has received funding from the European Community's Sixth Framework Programme as an Integrated Activity implemented as a Co-ordination Action. The text reflects only contractors' views and the European Community is not liable for any use that may be made of the information contained therein.

## Germination (expanded recommendations)

• Ideally, germination at the start of storage should be tested:
(1) before drying,
(2) after drying and before freezing,
(3) soon after banking and
(4) one year after freezing.
The results of the first two assess desiccation tolerance, the third gives an estimate of the initial viability at the time of entry to the cold store (provided dormancy has been removed) and the fourth one checks for any negative effect of cold storage. Realistically, because staff resources are likely to be limited, step three is felt to be most important and should be performed within one month of banking. Other tests should be included when the potential for desiccation or freezing problems for the species or genus are indicated in the literature.

• Germination tests should be recorded at frequent intervals if data on rate of germination is required, for instance on day 3, day 7 etc.

• Particular attention should be paid to the non-germinating seeds at the end of a test. The number of empty seeds (determined by a cut test) should be subtracted from the number of seeds sown to calculate percentage germination. Mouldy seeds and particularly those where the contents have become liquid or soft are a good indication that the seeds were not viable at the start of the test. However, when long incubation periods are used (for example, where species possess complex dormancy) there is a chance that some of these seeds may die during the germination test.

• If possible, abnormal germination (for example, radicles emerging but failing to elongate) should be recorded within germination tests; these give an early indication of seed ageing and signal the likelihood of poor performance if seedlings were to be raised for plant production.

• If the binomial distribution for seed germination is considered appropriate then only one sample per test is required. If an approximation to the normal distribution is used, then three or more samples per test should be used. In the latter case, tolerance tables can be used to identify samples that do not conform to the general response e.g., because the germination plate has become contaminated. For large collections, a sample size of 50 seeds is advisable. For collections with 101-500 seeds, 10 seeds per sample would give some indication of germination though little can be concluded statistically. For collections of 100 or less, tests on as few as five seeds could still provide helpful data.

**Figure 1** Germinating *Bromus bromoideus* on agar.
(© RBG, Kew)

• In order to avoid imbibition damage, seeds of susceptible species (for example, large seeded Fabaceae) should be gently rehydrated for 1-2 days in a saturated atmosphere (i.e., over water in a lidded container at room temperature) before testing.

• Seed germination strategies can vary considerably between species, populations, collection years and even between intervals of storage. Use published literature or databases such as RBGK's Seed Information Database (Liu *et al.*, 2008: http://data.kew.org/sid/sidsearch.html) and LEDA traitbase (http://www.leda-traitbase.org/tomcat/LEDAportal/index.jsp) as a guide. When there is no information (even about related species) the optimum temperature for germination and the likelihood of dormancy can be predicted from climate data and knowledge of seed structure and habitat. Various sources of climate data can be found online but WorldClim (www.worldclim.org) is particularly useful because it provides monthly rainfall and temperature data for any location by interpolation from nearest meteorological stations.

• There are two principal forms of seed dormancy: physical and physiological. Physical dormancy (PY) only occurs in about 17 plant families and is due to the seed coat being impermeable to water. PY can be removed by simple scarification of the seed coat by a variety of methods including: chipping, filing, drilling, rubbing with abrasive paper and brief exposure of dry seeds to high temperatures (>100 °C). Physiological dormancy (PD) on the other hand is a programmed shut down in embryo growth, which in most cases, serves to synchronise germination in the wild to a particular season in the future. Consequently, prolonged incubation at temperatures that mimic the passing of seasons (cold or warm stratification and dry after-ripening) are the most reliable ways of overcoming PD. In some cases PD can be overcome by subtle surgical treatments of the seed coat which remove a mechanical constraint to embryo growth. Such treatments may greatly reduce or even remove the need for stratification but require great care and precision to carry out successfully.

• The following concentrations of commonly used germination promoters are advised: 100 mg l-1 Potassium nitrate and 250 mg l-1 Gibberellic acid $GA^3$. If possible, Gibberellic acid should be used as a last resort for overcoming PD as continuous contact with $GA^3$ can lead to abnormal seedling growth. However, this risk can be reduced by exposing seeds to $GA^3$ only during the imbibition period (usually 2-3 days) and then transferring seeds to a plain water medium.

• The substrate for sowing could be agar, paper or sand. Agar (usually 1 %) has some advantages: requires low maintenance; has low risk of imbibition damage; maintains applied chemicals at constant concentration (as long as loss of water by evaporation is prevented by enclosing Petri dishes in a sealed container); allows white radicles to be visible on a dark background; and seedling plugs can be removed for transplanting. Care should be taken over selection of paper and proprietary germination papers are recommended. Sand has some advantages over other substrates for large seeds and seeds with a high microbial loading. Sand provides good isolation between germinated seeds minimising the spread of infection and it enables normal root development making it the best substrate for transferring seedlings to nursery medium for growing on.

**Figure 2** Placing Petri dishes of agar in closed plastic bags minimises moisture loss. (© RBG, Kew)

• In all cases deionised or distilled water should be used.

• The seeds should not be touching one another on the germination medium and sowing seeds in a grid gives a good visible check that the correct number of seeds have been sown.

• Germination Petri dishes should be sealed with parafilm or placed in closed plastic bags to minimise moisture loss.

• If possible, constant temperatures should be avoided except during stratification treatments. Continuous light should be avoided due to the risk of photo inhibition in susceptible species. The emission spectrum and photoperiod (hours light per 24 h) of lights used should be as close to natural conditions as possible. Lamps emitting natural daylight spectra should be used where possible. In most cases, a 8 or 12 hour photoperiod will be satisfactory. When diurnal alternating temperatures are used the amplitude should be around 10 °C and the higher temperature should correspond to the light period.

• An acceptable but lax criterion for a scoring seeds as germinated is when the radicles have emerged by 1-2 mm. A stricter criterion would include clear geotropism of the radicle and production of both epicotyl and radicle. Scoring seeds when both root and shoot have emerged will enable cases of epicotyl dormancy to be revealed and will give better correspondence with nursery performance when collections are being used for plant production.

• Labelling germination plates with barcodes is recommended provided good data can be entered onto the computer database at the laboratory bench.

**Further research**

• It is essential to remember that a germination test is not necessarily identical to a viability test because of non-germinating viable seeds. There should be further exploration on the use of triphenyl tetrazolium chloride and other topographical staining techniques and X-ray methods to distinguish between live and dead non-germinating seeds.

• The relationship between laboratory germination and nursery / field performance should be more closely assessed.

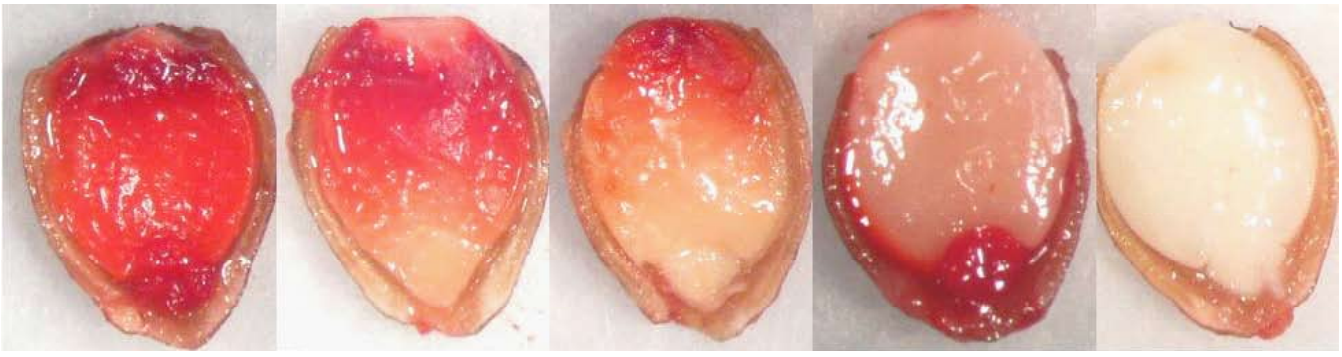

**Figure 3** Intrepreting the results of TZ tests to distinguish between live and dead non-germinating seeds is not always straightforward. (© RBG, Kew)