

Germination testing and quality

What lies behind an accession count

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Abstract

A collection is worth as much as the viable share of its samples. This paper describes the methods by which that share is determined — germination test, tetrazolium test, X-ray test and moisture determination —, names the typical sources of error and presents a simplified version that a conservation association or a school class can carry out without a laboratory. It is the basis for chapter 6 of the book and ties in with the early warning signs of paper P06.

1. Why testing is done

The FAO genebank standards tie three decisions to the germination test:

1. **Intake.** Initial germination should exceed 85 per cent for most crop species; lower values are acceptable for wild and forest species.
2. **Monitoring interval.** Retesting takes place after one third of the time predicted for viability to fall to 85 per cent of the initial value, or less, but no longer than 40 years. Where this time cannot be estimated and accessions are held at -18 °C in hermetically closed containers, a fallback interval of ten years for long-lived and five years or less for short-lived species applies (standard 4.3.3).
3. **Regeneration.** Below 85 per cent of the initial value, the accession is multiplied.

Without testing there is no initial value, no interval and no regeneration threshold. The finding from Bavaria's wild plant genebank shows what happens otherwise: in 21 per cent of the accessions examined, more than half the seeds were empty, infested or dead.

2. The germination test under the testing rules

The International Rules for Seed Testing of ISTA set out the procedure. Its main features:

Step	Specification
Sample size	400 seeds in four replicates of 100; smaller replicates for large seeds or scarce material
Substrate	between or on top of filter paper, in sand or substrate, depending on species
Conditions	species-specific temperature, light for many species; partly pre-treatment against dormancy
Duration	species-specific, with first and final count
Evaluation	normal seedlings, abnormal seedlings, hard seeds, fresh ungerminated seeds, dead seeds

Step	Specification
Result	share of normal seedlings, rounded to whole per cent, with permitted tolerance between replicates

Two points are often overlooked in practice.

Normal, not merely germinated. Only seedlings carrying all essential structures are counted. A seed that produces a root but forms deformed cotyledons is an abnormal seedling and does not count towards germination.

Ungerminated is not dead. Hard seeds (impermeable coat) and fresh ungerminated seeds (dormancy) are alive. They must be reported separately and clarified with a follow-up test. Adding them to the dead seeds underestimates viability — considerably so for wild species.

3. Supplementary methods

Method	What it shows	When it is useful
Tetrazolium test	living tissue stains red, dead tissue remains unstained	dormant seeds, clarifying ungerminated seeds, quick preliminary estimate
Cut test	firm, white embryo versus empty or rotten interior	simplest clarification of ungerminated seeds, even without a laboratory
X-ray test	fill, internal damage, infestation	before storing wild species, non-destructive
Moisture determination	seed moisture content as per cent of fresh weight	before storage; basis of any longevity calculation
Vigour test	germination speed, cold test, conductivity	early warning before germination falls

The tetrazolium test relies on living cells converting the colourless tetrazolium salt into a red dye. It delivers in a day what the germination test needs weeks for, but requires experience in interpreting the staining patterns.

Under the testing rules, moisture is determined in a drying oven using the method prescribed for the species: at a low constant temperature (103 degrees, 17 hours), mainly for oily species, or at a high constant temperature (130 to 133 degrees, one to four hours), for example for cereals. The loss of weight gives the moisture content. Without this figure the seed viability equation cannot be applied.

4. Sources of error

1. **Sample too small.** With 50 seeds, the uncertainty of a germination rate of 80 per cent is about plus or minus eleven percentage points (95 per cent range), with 400 seeds about plus or minus four. A lot lying close to the regeneration threshold cannot be classified reliably with a small sample.
2. **Wrong germination conditions.** A species that needs light or alternating temperature germinates poorly in the dark at constant temperature and appears old.
3. **Dormancy counted as death.** See section 2.
4. **Fungal infection in the substrate.** An infected seed infects its neighbours; dead seeds must be removed in time.
5. **Testing consumes material.** Every test costs seeds. For small accessions, the test quantity must be weighed against the remaining quantity; the standards require regeneration also when the remaining quantity is sufficient for fewer than three sowings.

The sampling uncertainty follows from the binomial distribution. For a measured germination rate p with n seeds, half the 95 per cent range is approximately 1.96 times the square root of $p(1 - p)/n$.

5. A simplified test without a laboratory

For conservation associations, seed libraries and schools, a simple procedure is sufficient, provided it is always carried out the same way and documented:

1. **Sample:** 2 times 50 seeds, drawn at random from the lot.
2. **Substrate:** two layers of kitchen paper or filter paper in a flat box with a lid, evenly moist, not wet.
3. **Conditions:** room temperature around 20 degrees; light for species that need it, otherwise dark.
4. **Count:** after 7 and after 14 days; seedlings with a healthy root and unfolded cotyledons are counted.
5. **Follow-up:** cut open ungerminated seeds. Firm and white means dormant, empty or soft means dead.
6. **Record:** date, sample, both counts, cut test result, germination rate. The storage register in the templates of the appendix has a row for this.

The result is coarser than a test under the rules — with 100 seeds the uncertainty around 80 per cent germination is about plus or minus eight percentage points —, but it fulfils the actual purpose: it shows whether a lot is still alive and whether it is approaching the regeneration threshold.

6. Three figures instead of one

The most important reporting rule of the strand follows from testing: **number of accessions, viable holdings and duplicated holdings are to be shown separately** (papers P04 and P06). A collection that only counts does not know what it has.

Limits of this paper

The paper summarises the testing methods; it does not replace the International Rules for Seed Testing, whose species-specific specifications on substrate, temperature, duration and tolerances are authoritative. The simplified test is a self-check procedure and not a recognised testing method; its results cannot be used for marketing. The figures on sampling uncertainty are approximations based on the normal distribution.

References

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7. Papers P02, P04, P06 and P08 of this strand.