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Science for resilient livelihoods in dry areas

Seed Viability

CWANA Workshop on Conservation and Use of Plant Genetic Resources

5-16 December 2022

What is seed viability?

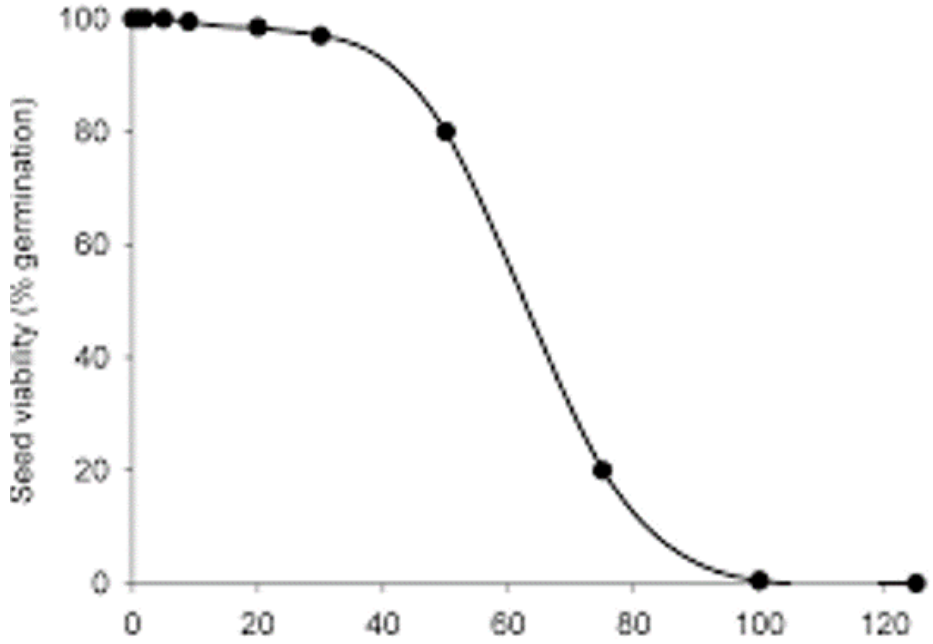
Definition: Seed viability is the measure of how many seeds in a lot are alive and could develop into plants that will reproduce under appropriate field conditions.

Why should seed viability be determined?

- Seeds should be capable of producing plants when sown in the field.
- Seeds must have high viability at the start of storage and maintain it during storage.
- Seeds with a high initial viability will also survive longer in storage.

What is seed viability?

- Seed viability declines slowly at first and then rapidly as seeds age.
- It is important to know when this decline occurs in order to take action to regenerate the accession.
- Excessive deterioration will lead to loss of material



When should viability be determined?

1. Before seeds are packaged and placed in the genebank
2. at regular intervals during storage.

- Storage of seeds with high initial quality will maximize accession longevity.
- Monitoring viability during storage facilitates timely identification of accessions that require regeneration to ensure continued availability of conserved germplasm.

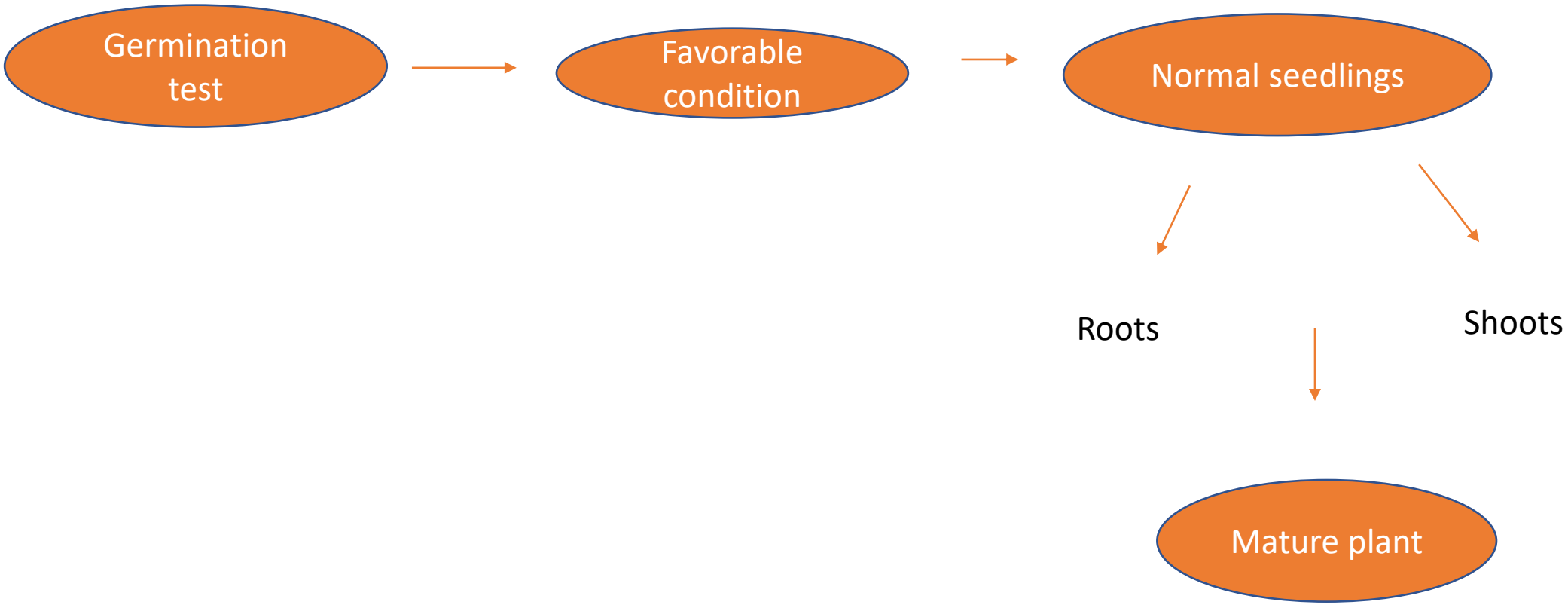
How should viability be determined?

- Germination test.
- Biochemical tests

have the advantage of being quicker but are not as accurate as the germination test.

Tetrazolium test

What is a germination test?



How is germination tested?

- Basic requirements : water, oxygen, light and suitable temperature.
- Seeds of different species have different requirements, and no general set of conditions can be relied upon to germinate seeds of all species.
- Seeds of some species are more tolerant and germinate in a wide range of conditions, but complete germination can only be achieved under optimum conditions.

How many seeds should be tested?

- Standard protocols as outlined by the International Seed Testing Association (ISTA) recommend two replicates with 100 seeds per replicate.

- If the test results show that germination is below 90%, an additional 200 seeds should be tested using the same method.

For species with large seeds that have a low seed-multiplication rate and wild species, two replicates of 50 or 25 seeds each can be used, depending on the available quantity.

- Overall seed viability is taken as the mean of the two tests.

- Germination is not complete until the seedling can be judged as normal.

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Germination testing

Step 1: Preparation for germination testing

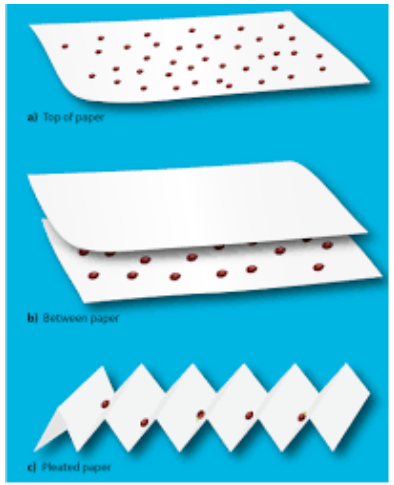
1. Check the specific requirements for temperature, light and any other treatments needed to test germination of a species.
2. Check that the equipment and appropriate environment are available to fulfil these requirements. If not, the best possible alternatives should be found.
3. Take a random sample of seeds.
4. Count out 200 seeds (or fewer, depending on availability) for each accession.
5. Divide these seeds into at least two replicates.
6. If the seeds are very dry (with moisture content below 8%) and are likely to suffer from imbibition damage, it may be necessary to raise the moisture content (this process is called *humidification*) to 15–17% before testing for germination

Seeds of wild species with hard seed coats may require scarification (puncture the seed coat with a razor blade, sandpaper or scalpel without damaging the embryo) before humidification or sowing.

Step 2: Setting up the germination test

Four methods described below are suggested:

1. Top-of-paper method
2. Between-paper method
3. Germination in sand
4. Agar method



Absorbent paper (filter paper) is used as substrate for germination in the first two methods.

Paper substrate quality

It is important that high-quality paper be used as a substrate in order to obtain uniform germination and reproducible results.

- The paper used as substrate should not be toxic to developing seedlings.
- It should be able to absorb and supply sufficient moisture for the seeds to germinate.
- It should be strong enough not to disintegrate when handled, and not to be penetrated by the roots of developing seedlings.
- It should have a neutral pH of 6–7.

Controlling fungi in germination tests

1. Clean and disinfect (by surface sterilization with 70–95% alcohol or 20% domestic bleach) the test area and incubators between batches
2. Space seeds properly and ensure that individual seeds do not touch each other.
3. Provide an optimum environment for germination so that seeds germinate quickly
4. Ensure cleanliness of germination test media and containers
5. Avoid imbibition injury that could lead to leakage of cell contents, which provide sources of nutrients to fungi.
6. Promptly remove decaying seeds to prevent the spread of fungi to neighboring seeds.
7. Remove seed-covering structures (such as glumes) before tests when they are found to be sources of infection.

Top-of-paper method

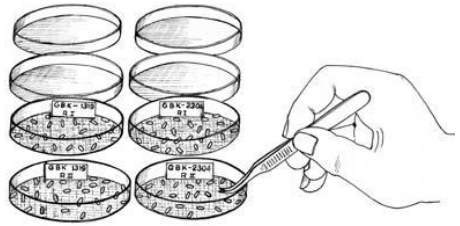
- Most suitable for species with seeds smaller than 2 mm in diameter such as small-seeded vegetables and forage grasses.
- The seeds are germinated on top of moist absorbent paper in containers with close-fitting lids to prevent moisture loss.
- Commonly used containers include 9 cm glass or plastic Petri dishes.

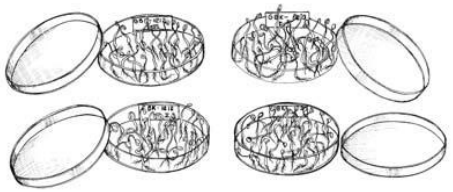
Top-of-paper method

1. Sterilize container surfaces by wiping with 70–95% alcohol or soaking in 20% bleach or hot water at 55°C for 10–15 minutes.
2. Place the paper substrate at the bottom of the container or Petri dish.
3. Label containers with accession number, number of replicate and testing date; use a pencil or permanent marker for labelling, or barcode label if this possible.
4. Add the required volume of distilled water.
   - If distilled water is not available, boil and cool tap water
   - The volume of distilled water depends on the thickness of the paper substrate and the size of container.
5. Spread the seeds uniformly on the absorbent paper.

Top-of-paper method

6. Cover the containers and ensure that there is no air lock.
7. Place the containers in a germinator or incubator maintained at the recommended temperature for germination of the species
8. Check the moisture level of the substrate regularly.
9. Run the test for the recommended period.
10. If some seeds have not germinated and appear to be dormant, treat to break dormancy.
11. take notes





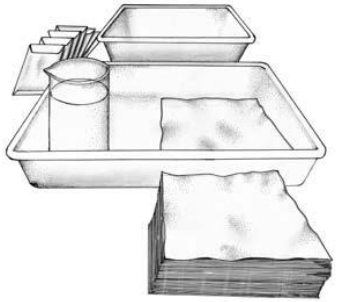
Between-paper method

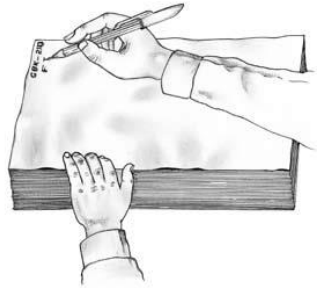
- This method is most appropriate for species with medium and large seeds between 2 mm and 1 cm in diameter, including many cereals, grain legumes and vegetables.
- Seeds are germinated between two layers of moist paper towel.
- The towels should meet the specifications described above
  (e.g., non-toxic paper toweling from Seedburo Equipment Co., regular and heavy weight germination paper from Hoffman Manufacturing, Inc. and Grade 3663 seed testing paper from Whatman Plc.).

The between-paper method is cheap and easy to prepare, but the seeds cannot be observed without unrolling the paper.

Do not dry and reuse the paper for another test as it could carry fungal contamination from one test to another.

Between-paper method

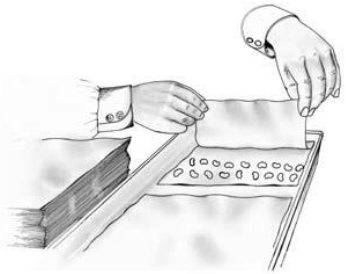




1. Choose the absorbent paper.
2. Label the paper
3. Moisten the paper with distilled water
4. Arrange the seeds in rows at regular intervals.

About 4 cm from the top edge, leaving a 3-4 cm gap on the sides.

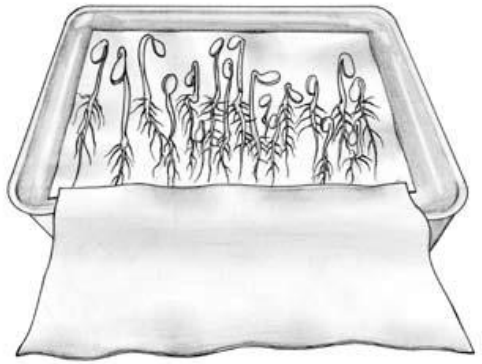
The distance between seeds should at least three to five times the seed diameter.



5. Cover the seeds with another sheet of moist paper towel.
6. Roll the paper loosely
7. Use a paper clip or rubber band to hold the rolled papers and prevent them from falling apart.

Between-paper method

8. Keep the rolls upright in a deep-bottom plastic tray.
9. Add a sufficient quantity of water to the tray
10. Place the tray in an incubator or germinator
11. Keep the towels moist by spraying with water
12. Count the germinated seeds by unrolling the paper carefully to avoid tearing it or damaging the roots of young seedlings.
13. If some seeds have not germinated and appear to be dormant, treat appropriately.
14. take notes



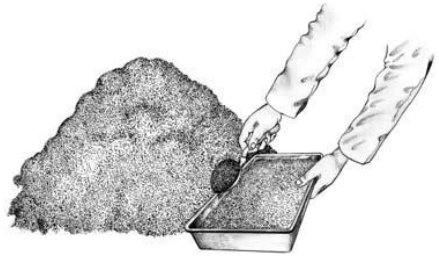
Germination in sand

For large seeds (with a diameter greater than 1 cm)

- Difficult to germinate in Petri dishes
- Too heavy for the between-paper method.

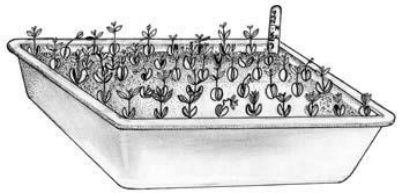
Germination in sand

1. Pack sterile, moist sand into pots or deep-bottom plastic trays with drainage.
2. Water the sand until it is moist. Do not use excess water.
3. Make holes in a regular equidistant pattern at about the same depth as the size of the seeds.
4. Prepare a plastic or wooden label with the accession number, date of sowing and replicate number, and place it in each tray.
5. Place one seed in each hole and cover the holes with sand.

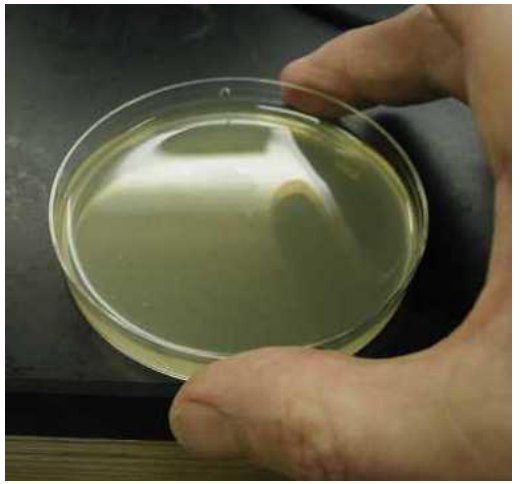


Germination in sand

6. Water the sand again by sprinkling to ensure that the sand layer is not displaced, or the seeds are not disturbed when watering.
7. Place the trays in appropriate light and temperature for the species.
8. Keep the substrate moist during tests by adding water, but do not over-water.
9. Run the test for the period recommended for the species and count the number of seeds that have sprouted.



Agar method



Agar is an alternative substrate to paper,

- for testing germination in small and medium-sized seeds.

1. Sterilize the surface of the containers
2. Label with accession number, number of replicate and testing date.
3. Prepare 1% agar solution
    - It is susceptible to contamination from airborne fungi and bacteria,
4. Arrange the seeds equidistantly on the surface of the agar.
5. Cover the dishes with their lids and place them in an incubator
6. Run the test for the recommended period and count the number of seeds that have germinated

Step 3: Evaluation of germination tests

1. Seedlings removed during germination testing:
    - A. Normal seedlings possess adequate root and shoot structures, which are essential for further development into plants.
    - B. Abnormal seedlings are incapable of further development and suffer deficiency, decay or weakness in their root or shoot systems.
2. Observe regularly and remove normal and abnormal germinant to allow a less-crowded environment;
    - remove fungi-infected seeds in order to prevent the spread of infection.
    - initial germination count after 4,7,10,14 and 21 days.
    - Some species (grasses) count at 14 days and at 21 or 28 days
3. Record observations: normal, abnormal, dead.. Ec..

Step 3: Evaluation of germination tests

4. Count and record all un-germinated and dead seeds in each replicate.
5. Calculate the mean percentage germination of the accession from the results of all the replicates
6. Repeat the germination test if the difference between the two replicates exceeds 10%
7. Once a seed has been germinated, the resulting seedling can be discarded or transplanted for regeneration when the number of seeds in storage is critically low.

Why do some seeds fail to germinate?

- Seeds fail to germinate either because they are dead or dormant.

To determine if seeds are dead or dormant, inspect the un-germinated seeds with a pair of tweezers to establish whether they are soft or firm.

- Dead seeds usually soften and rot
- Viable seeds are un-germinated with firm embryos
    - A high percentage of these seeds indicates that germination conditions were not optimal or that seeds are dormant.
    - **common in freshly harvested seeds and in many wild species of crop plants.**

Dormancy

Dormancy refers to the state in which viable seeds fail to germinate even under conditions normally favorable for germination.

Types of dormancy

1. Seed-coat dormancy

Physical, chemical or mechanical conditions prevent uptake of moisture.

2. Embryo dormancy

Inhibiting substances usually within the embryo or surrounding tissues prevent germination.

In certain species, seed embryos are underdeveloped or not fully formed at seed dispersal. In these species, the embryo continues to grow after dispersal, and germination is prevented until the embryo reaches a species-specific critical length.

For germination to occur, both types of dormancy must be broken.

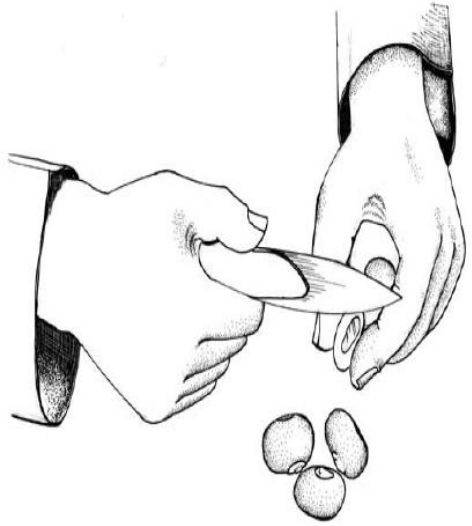
Breaking seed-coat dormancy

1. Puncturing / scarifying the seed coat by piercing, nicking, chipping or filing with a knife, needle or sandpaper

Manual scarification is effective at any point on the seed coat,

avoid the micropylar region as it is the most sensitive part of the seed where the radicle is

If seed-covering structures prevent growth of the embryo, remove them to allow germination.



2. Leaching of seed coat inhibitors

- by placing the seed under running water for several hours
- by soaking the seed in a large volume of water that is changed every six to 12 hours.
- By using concentrated sulphuric acid for 2–45 minutes

To remove the waxy covering, place the seeds in water at 75°C for three to six minutes.

Do not boil the seeds.

Breaking embryo dormancy

Pre-chilling (cold stratification)

Seeds are placed on a moistened germination substrate and kept at 3° to 5°C in a refrigerator for 7-14 days.

Preheating

at a temperature not exceeding 40˚C for up to seven days with free air circulation

Gibberellic acid

Germination test paper is moistened with a 0.05% solution of gibberellic acid

Potassium nitrate

A 0.2% solution of potassium nitrate (KNO3)

Light

the tests should be illuminated for at least eight hours of every 24-hour cycle (light should coincide with the high-temperature cycle.)

Documentation

- Number of seeds tested per replicate
- Number of replicates
- Method of germination testing
- Date of germination testing
- Duration of testing (or days of first and final counts)
- Number of germinated seeds at first count
- Dormant/hard seeds at first count (%)
- Special treatments for dormancy breaking (if any)
- Final germination (% normal seedlings)
- Abnormal germination (%)
- Dead seeds (%)
- Tolerance levels for statistical accuracy

Documentation

Plot	Ig	Crop	Expt	Ori	Tax_name	Vt-number	Rep_number	nb_Seeds	Day 4		Day 7		Day 10		Day 14		Total_germ	%_germ	Total_non germ	Acc_no	Store	Vt_number	Pretreatment_1	Pretreatment_2	Substrate	Chemical	Temperature	Light	date sown	Nb_replicates	Germ_average	Reps_in_tolerance
									R 1	R 2	R 1	R 2	R 1	R 2	R 1	R 2																

Thank you