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.

5. SEED QUALITY TESTING

5.1 Seed viability testing

What is seed viability?

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?

It is very important that seeds stored in the genebank are capable of producing plants when sown in the field. They 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. 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?

Viability of accessions should be determined:

- before seeds are packaged and placed in the genebank; and
- at regular intervals during storage.

Viability-testing can take from a few days to weeks, depending on the species. If possible, the results of viability tests should be made available before seeds are packaged and placed in the genebank so that poor-quality seeds can be identified and regenerated.

While awaiting the results of viability tests or if there is a delay in conducting the viability tests before storage, seeds should be placed in a cool environment to minimize their deterioration.

How should viability be determined?

Many different methods are available to test seed viability. The most accurate and reliable method is the germination test. There are also biochemical tests, which have the advantage of being quicker, but are not as accurate as the germination test.

They also require special skills to conduct and interpret. These tests are not usually recommended for general use to test for seed viability in genebanks.

What is a germination test?

A germination test is performed to determine what proportion of seeds in an accession will germinate under favourable conditions and produce normal seedlings (seedlings that have the essential structures—roots, shoots and sufficient food reserves) capable of development into reproductively mature plants (see Flowchart 5.1).

How is germination tested?

Basic requirements for seed germination are: 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?

- A fixed-sample size germination test using 200 seeds is recommended to determine viability at the beginning of storage.
- The International Standards for Genebanks (FAO/IPGRI, 1994) recommend using a minimum of 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. Overall seed viability is taken as the mean of the two tests.

For species with large seeds that have a low seed-multiplication rate and those with problems in seed regeneration (such as wild species), it may become difficult to expend 200 seeds for a germination tests. In this case, two replicates of 50 or 25 seeds each can be used, depending on the available quantity.

When has a seed germinated?

Seed germination can be defined as the resumption of growth of the embryo and emergence or protrusion of the radicle from the covering structures. For seed testing in genebanks, germination is not complete until the seedling can be judged as normal according to specific criteria for each species (see Association of Seed Analysts [AOSA], 2005; ISTA, 2005). This is because the intent of seed testing is to give an indication of how the seeds will perform as propagules in the field.

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 (see Table 5.1).
2. Check that the equipment and appropriate environment are available to fulfil these requirements. If not, the best possible alternatives should be found.

Flowchart 5.1. Germination testing.

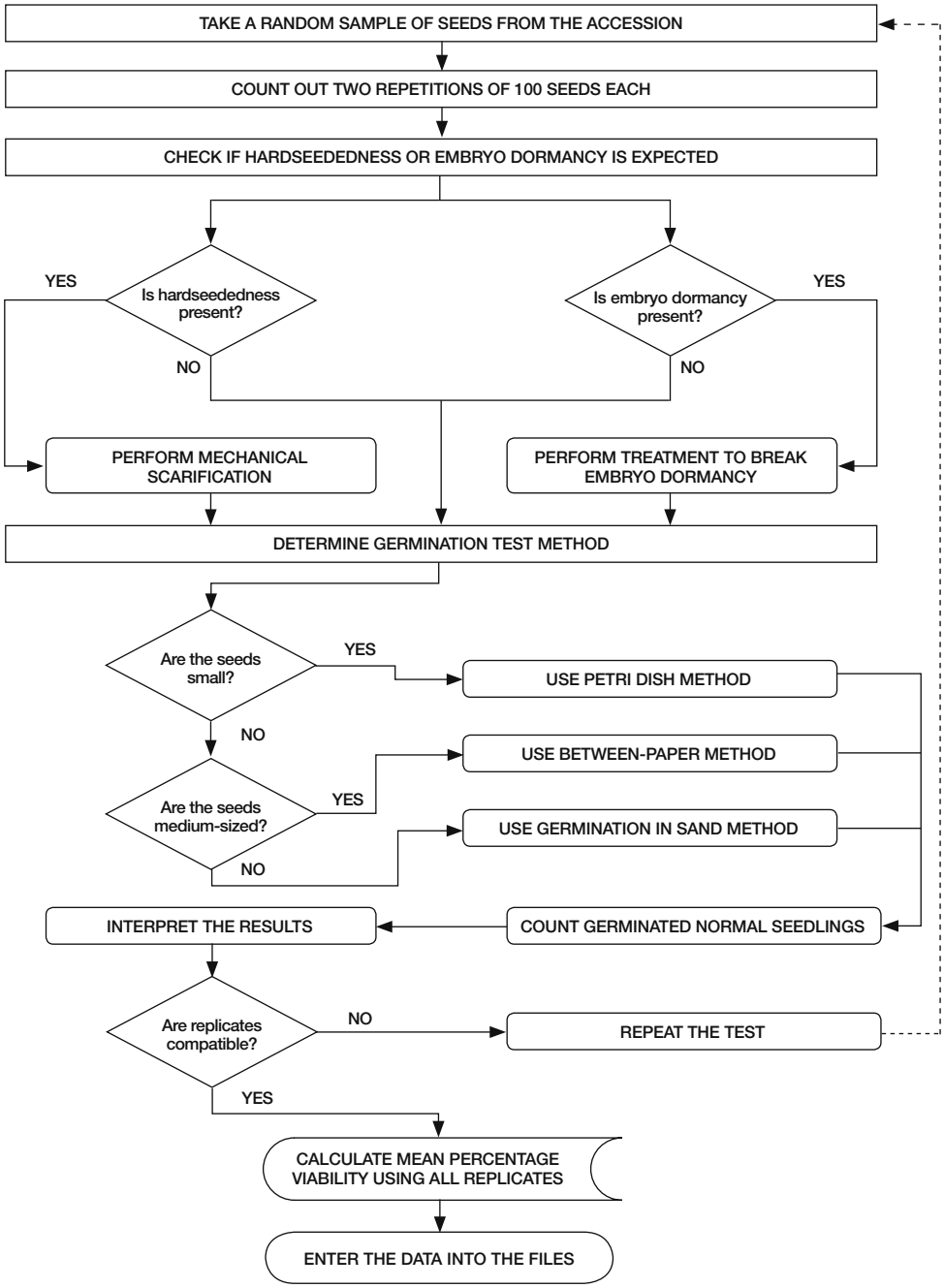


Table 5.1. Guidelines for testing germination of the most common crop species. Refer to ISTA (2005) or AOSA (2005) for information on other crops.

Crop	Species	Substrate*	Temp (°C)**	First, Final count (days)	Special treatments; Additional directions for fresh and dormant seeds
Alfalfa	*Medicago sativa*	TP; BP	20	4, 7	Mechanical scarification of hard seeds
Annual rape	*Brassica napus*	BP, TP	20/30	3, 7	
Barley	*Hordeum vulgare*	BP; S	20	4, 7	Pre-chill at 5°C or 10°C for five days
Bermuda grass	*Cynodon dactylon*	TP	20/30	7, 21	Light; KNO_3
Berseem	*Trifolium alexandrinum*	TP; BP	20	3, 7	
Black gram	*Vigna mungo*	BP	20/30; 25; 20	3, 7	
Black mustard	*Brassica nigra*	TP; BP	20/30; 20	3, 7	Light; KNO_3 and pre-chill at 10°C for three days
Bottle gourd	*Lagenaria siceraria*	BP; S	20/30; 20	14	
Buck wheat	*Fagopyrum esculentum*	BP; TP	20/30; 20	3, 6	
Cabbage	*Brassica oleracea* var. *capitata*	TP; BP	20/30; 20	3, 10	Pre-chill at 5° or 10°C for three days; KNO_3 and light
Carrot	*Daucus carota*	TP; BP	20/30; 20	6, 14	GA_3 50 ppm
Castor bean	*Ricinus communis*	BP; S	20/30	7, 14	
Cauliflower	*Brassica oleracea* var. *botrytis*	TP; BP	20/30; 20	3, 10	Pre-chill at 5° or 10°C for three days; KNO_3 and light
Chickpea	*Cicer arietinum*	BP	20	5, 8	Mechanical scarification of hard seeds
Chicory	*Cichorium intybus*	TP	20; 20/30	5, 14	Light; KNO_3
Coriander	*Coriandrum sativum*	TP; BP	15	6, 21	
Cotton	*Gossypium* spp.	BP; S	20/30; 25	4, 12	De-linting; Mechanical scarification of hard seeds
Cowpea	*Vigna unguiculata*	BP; S	20/30; 25	5, 8	
Cucumber	*Cucumis sativus*	TP; BP	20/30	3, 7	Keep substrate on dry side
Eggplant	*Solanum melongena*	TP; BP; S	20/30	7, 14	Light; KNO_3
Faba bean	*Vicia faba*	BP; S	20	4, 14	Pre-chill at 10°C for three days
Field bean	*Phaseolus vulgaris*	BP; S	20/30; 25; 20	5, 8	

Crop	Species	Substrate*	Temp (°C)**	First, Final count (days)	Special treatments; Additional directions for fresh and dormant seeds
Finger millet	*Eleusine corocana*	TP	20/30	8	KNO_3
Flax	*Linum usitatissimum*	BP; TP	20/30; 20	3, 7	
Foxtail millet	*Setaria italica*	TP	20/30	4, 10	
Grain Amaranth	*Amaranthus* spp.	TP	20/30; 20	7, 14	
Grass pea	*Lathyrus sativus*	BP; S	20	4, 14	Mechanical scarification of hard seeds
Hot pepper	*Capsicum frutescens*	TP; BP	20/30	6, 14	Light and KNO_3
Indian mustard	*Brassica juncea*	TP; BP	20/30	3, 7	Light; Pre-chill at 10°C for seven days and test for five additional days
Lentil	*Lens culinaris*	BP, S	20	5, 10	Mechanical scarification of hard seeds
Lettuce	*Latuca sativa*	TP; BP	20	7	Light; pre-chill
Lima bean	*Phaseolus lunatus*	BP; S	20/30; 25	5, 9	
Lupin	*Lupinus angustifolius; L. albus*	BP; S	20	3, 10	
Maize	*Zea mays*	BP; S	20/30; 25; 20	4, 7	
Mung bean	*Vigna radiata*	BP; S	20/30; 25	3, 7	
Muskmelon	*Cucumis melo*	BP; S	20/30	4, 10	Keep substrate on dry side
Oat	*Avena sativa*	BP; S	20	5, 10	Pre-chill at 5°C or 10°C for five days and test for ten days
Okra	*Abelmoschus esculentus*	BP; TP	20/30	4, 14	
Onion	*Allium cepa*	BP; TP	20	6, 10	
Orchardgrass	*Dactylis glomerata*	TP	15/25	7, 21	Light; Pre-chill at 5°C or 10°C for seven days
Pea	*Pisum sativum*	BP; S	20	8	
Peanut	*Arachis hypogaea*	BP; S	20/30; 25	5, 10	Ethephon, 0.2%
Pearl millet	*Pennisetum glaucum*	TP; BP	20/30; 25	3, 7	
Pigeonpea	*Cajanus cajan*	BP	25	5, 10	Mechanical scarification of hard seeds

Crop	Species	Substrate*	Temp (°C)**	First, Final count (days)	Special treatments; Additional directions for fresh and dormant seeds
Potato	*Solanum tuberosum*	TP; BP	20/30; 20	8, 16	GA_3, 2000 ppm
Pumpkin	*Cucurbita maxima*	BP; S	20/30; 25	4, 7	Keep substrate on dry side
Radish	*Raphanus sativus*	TP; BP	20/30; 20	4, 6	
Red clover	*Trifolium pratense*	TP; BP	20	4, 10	Pre-chill at 5°C or 10°C for five days
Rice	*Oryza sativa*	TP; BP; S	20/30; 25	5, 14	Preheat at 40°C for five days
Rye	*Secale cereale*	TP; BP; S	20	4, 7	Pre-chill at 5°C or 10°C for five days
Rye grass	*Lolium perenne*	TP	15/25; 20	5, 14	KNO_3 and pre-chill at 5°C or 10°C for five days
Safflower	*Carthamus tinctorius*	TP; BP	20; 25;	4, 14	Light at 15°C
Sesame	*Sesamum indicum*	TP	20/30	3, 6	
Sorghum	*Sorghum bicolor*	TP; BP	20/30; 25	3, 10	Pre-chill at 5°C or 10°C for five days
Soya bean	*Glycine max*	BP; S	20/30; 25	5, 8	
Squash	*Cucurbita pepo; C. moschata*	BP; S	20/30	4, 7	Keep substrate on dry side
Strawberry	*Fragaria ananassa*	TP	20/30; 20	28	Light
Sugar beet	*Beta vulgaris* subsp. *vulgaris*	TP, BP, S	20/30; 20	3, 10	Pre-wash and dry at a maximum of 25°C
Sunflower	*Helianthus annuus*	BP; S	20/30; 25; 20	3, 7	
Sweet clover	*Melilotus albus*	TP; BP	20	4, 7	
Timothy grass	*Phleum pratensis*	TP	20/30	5, 10	Light; KNO_3 and Pre-chill at 5°C or 10°C for five days
Tobacco	*Nicotiana tabacum*	TP	20/30	4, 14	Light
Tomato	*Lycopersicon esculentum*	TP; BP	20/30	5, 14	Light; KNO_3
Triticale	*Triticosecale*	TP; BP; S	20	4, 7	Pre-chill at 5°C or 10°C for five days
Vetch	*Vicia sativa*	BP; S	20	5, 10	
Watermelon	*Citrullus lanatus*	BP; S	20/30; 25	4, 14	Keep substrate on dry side; Test at 30°C
Wheat	*Triticum aestivum*	TP; BP; S	20	4, 7	Pre-heat (30°–35°C); Pre-chill; GA_3
White clover	*Trifolium repens*	TP; S	20	3, 10	Pre-chill at 5°C or 10°C for five days

*TP = Top of paper, BP = Between paper, S = Sand
**20/30 = alternating temperatures of 20°C applied for eight hours per day and 30°C for 16 hours.

3. Take a random sample of seeds from each accession after gently mixing the seed lot in its container or by spreading them on a clean surface and mixing thoroughly.
4. Count out 200 seeds (or fewer, depending on availability) for each test. Return excess seeds back to the container.
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 (see Box 5.1).

Box 5.1. Humidification of dry seeds.

Small seeds

1. Spread the seeds uniformly on a petri dish.
2. Place three very moist paper towels flat inside a large polythene box.
3. Place the petri dishes (without covers) containing seeds on top of the moist paper and close the box with a tight-fitting lid.
4. Place the box in an incubator at 20°C for 24 hours or more, depending on the initial moisture content.

Large seeds

1. Place the seeds in porous bags made of mosquito netting or similar material.
2. Place the bags on top of a gauge above water in a desiccator. Care should be taken to avoid direct contact of seeds with water.
3. Place the desiccator at 20°C for about 48 hours. The seed layer should not be more than one seed deep to enable all seeds to absorb moisture equally from the atmosphere.

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

Although several methods are available to test germination, the four methods described below are suggested; they can be used for most species and give uniform results:

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

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

All new batches of paper substrate should be tested for quality upon receipt.

Paper substrate quality

It is important that high-quality paper be used as a substrate in order to obtain uniform germination and reproducible results. If possible, the paper should meet the following specifications (ISTA, 2005):

- The paper used as substrate[10] 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.

Simple test for paper quality

A. Presence of toxic substances

1. Cut the paper to size and place in a 9–cm Petri dish.
2. Moisten the paper with sufficient water.
3. Test using seeds of sensitive species like Bermuda grass (*Cynodon dactylon*), petunia (*Petunia hybrida)* or tobacco (*Nicotiana tabacum*) to observe germination on the moistened paper.
4. Evaluate root development after five days.
   - Symptoms of paper toxicity include shortened and discoloured root tips.

B. Paper strength

1. Moisten the paper and hold it in the air from one corner.
   - The paper should not fall apart.

C. Moisture absorption

1. Cut the paper into 10-mm wide strips.
2. Hold vertically with about 20 mm of the paper immersed in water.
3. Measure the height above the level that the moisture has risen to.
   - The minimum standard is a 30 mm rise in two minutes.

Controlling fungi in germination tests

Fungal contamination is a common occurrence during germination testing, especially with legume seeds; it is usually associated with immature, damaged or old seeds. It can also arise during pre-treatments like seed extraction or as a result of hygiene problems in the seed-testing area. Adopt the following laboratory practices to minimize the risk of fungal contamination:

1. Clean and disinfect (by surface sterilization with 70–95% alcohol or 20% domestic bleach) the test area and incubators between batches to limit the spread of fungal attack; washing hands, benches and inside incubators with hot soapy water is a simple but effective technique to reduce contamination.

[10] Examples of standard substrates include grade 181 filter paper from Whatman and 400PT non-toxic paper towels from Seedburo.

2. Space seeds properly and ensure that individual seeds do not touch each other. Use greater numbers of replicates, if necessary.
3. Provide an optimum environment for germination so that seeds germinate quickly—the temperature regime should be suitable and the test environment must be well-aerated.
4. Ensure cleanliness of germination test media and containers—make sure that they are not sources of inoculum. Sterilize container surfaces by wiping them with 70–95% alcohol or soaking them in 20% bleach or hot water at 55°C for 10–15 minutes.
5. Avoid imbibition injury (by prior humidification of the seeds) 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 neighbouring seeds. If contamination is increasing, wash the seeds well in 1–10% bleach and re-commence the test in a clean container on a new substrate.
7. Remove seed-covering structures (such as glumes) before tests when they are found to be sources of infection.
8. Remove seeds that have germinated before harvest and subsequently dried, as they can be sources of infection.

While these practices minimize the risk of fungal contamination, dressing seeds with fungicides such as Thiram or Benlate, or sterilizing surfaces with sodium hypochlorite reduces fungal attack during germination testing. Use of fungicides may affect the results of germination tests and may constitute a health hazard to seed analysts, however. They should be used only when essential, but are extremely useful during field sowing for regeneration.

Seed dressing

1. Add a pinch of fungicide to the container holding seeds prepared for germination testing.
2. Thoroughly shake the container so that seeds receive a uniform coating of fungicide.

Surface sterilization

1. Soak seeds for ten minutes in a 1% solution of sodium hypochlorite. The concentration of household bleach is usually 5% sodium hypochlorite. Add 80 ml of distilled water to 20 ml of bleach to get a 1% solution.
2. Rinse the seeds thoroughly before testing for germination.

Top-of-paper method

This method is 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.

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. Cut the absorbent paper to the size and shape of the container. For Petri dishes, round filter paper such as Whatmann Grade 181 of appropriate diameter can be used.
3. Place the paper substrate at the bottom of the container or Petri dish.
4. Label containers with accession number, number of replicate and testing date; use a pencil or permanent marker for labelling.
5. Add the required volume of distilled water. If distilled water is not available, boiled and cooled tap water can be used. The volume of distilled water depends on the thickness of the paper substrate and the size of container. For Whatman Grade 181 filter paper in 9 cm Petri dishes, 4 ml of water is required. The filter paper should not be so wet that a film of water forms around the finger when it is pressed.
6. Firm down the paper substrate in the container using an upside-down funnel or tweezers.
7. Spread the seeds uniformly on the surface of the paper so that they are not touching. It is recommended that the distance between seeds should be at least three to five times the seed diameter.
8. Cover the containers and ensure that there is no air lock resulting from excess moisture on the covers.
9. Place the containers in a germinator or incubator maintained at the recommended temperature for germination of the species (see Table 5.1).
10. Check the moisture level of the substrate regularly, especially when humidity inside the cabinets is not controlled or when the temperature is set at 25°–30°C. Blotters usually need to be watered several times during the test. Alternatively, keep the containers in a thin plastic bag (loosely folded at the open end, but not sealed to allow diffusion of oxygen) to prevent the substrate from drying.
11. Run the test for the recommended period (see Table 5.1) and count the number of seeds that have germinated.
12. If some seeds have not germinated and appear to be dormant, treat with appropriate techniques to stimulate germination (see Table 5.1) and continue the test until all seeds have germinated or until no further germination has occurred after two consecutive counts.
13. Make a note of the seeds that did not germinate but are firm and sound at the end of the first count, and those that failed to germinate and are presumed dead at the end of the germination test.

Figure 5.1 shows the stages in testing germination using blotters in Petri dishes.

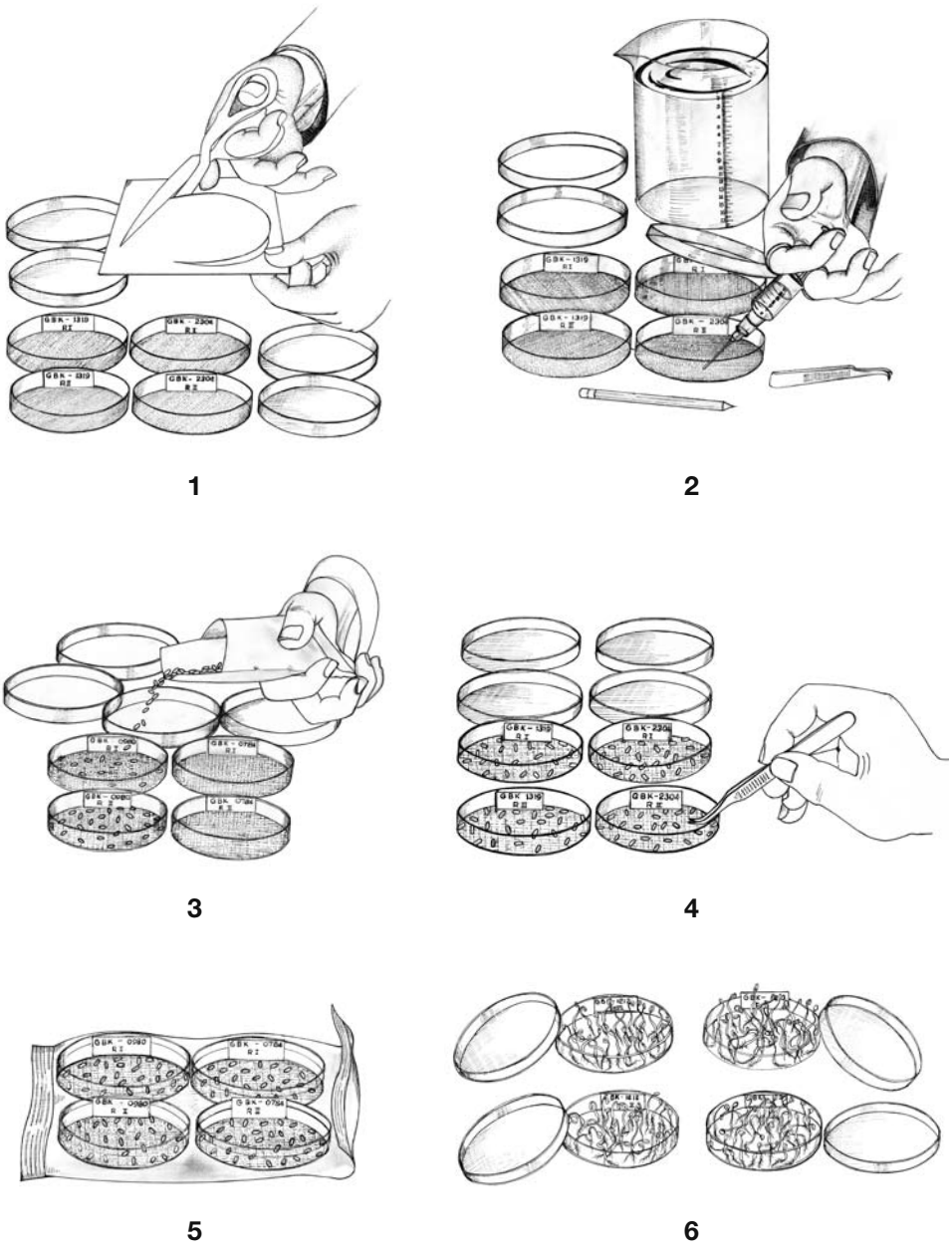


Figure 5.1. Seed germination testing on top of absorbent paper in Petri dishes.

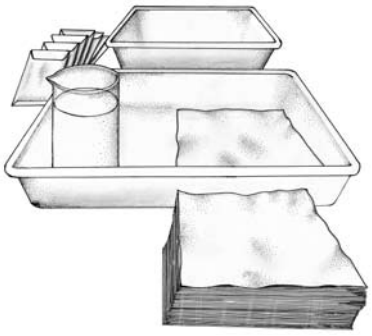
Between-paper method

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. Where possible, the towels should meet the specifications described above (e.g., non-toxic paper towelling from Seedburo Equipment Co., regular and heavy weight germination paper from Hoffman Manufacturing, Inc. and Grade 3663 seed testing paper from Whatman Plc.).

1. Cut the paper to a convenient size to hold one replicate of the seeds.
2. Label the paper at one end with the accession number, replicate number and the testing date. Use a pencil or permanent marker for labelling.
3. Moisten the paper with 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. Ideally, the distance between seeds should be 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 from opposite the label end.
7. Use a paper clip or rubber band to hold the rolled papers and prevent them from falling apart.
8. Keep the rolls upright in a deep-bottom plastic tray.
9. Add a sufficient quantity of water to the tray (covering the bottom 3 cm of rolls).
10. Place the tray in an incubator or germinator maintained at the recommended temperature and run the test for the recommended period (see Table 5.1).
11. Keep the towels moist by spraying with water (use spray bottles) if necessary, especially when temperatures are high (25°–30°C).
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 with an appropriate technique to stimulate germination (see Table 5.1). Continue the test until all seeds have germinated or until no further germination has occurred after two consecutive counts.
14. Make a note of the seeds that did not germinate but are firm and sound at the end of first count, and those that failed to germinate and are presumed dead at the end of the germination test.

Figure 5.2 shows the stages in preparation of germination tests using paper towels.

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.



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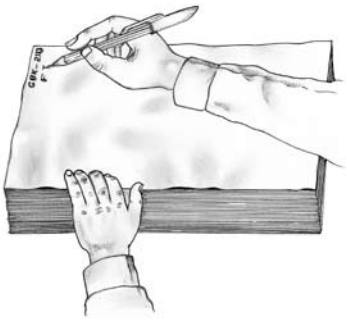

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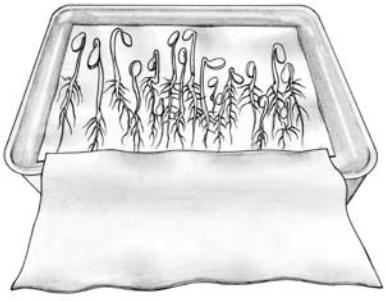

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Figure 5.2. Seed germination testing by between-paper method.

Germination in sand

This method is most appropriate for large seeds (with a diameter greater than 1 cm), which are difficult to germinate in Petri dishes or too heavy for the between-paper method.

1. Pack sterile, moist sand into pots or deep-bottom plastic trays with drainage. A single sheet of paper can be placed in the base of the tray to keep the sand from pouring out through the drainage holes.
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. Ideally, the distance between holes should be at least three to five times the seed diameter.
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.
6. Water the sand again by sprinkling to ensure that the sand layer is not displaced or the seeds are not disturbed when watering. Bottom-watering is better than top-watering—it is achieved by placing the test containers in larger plastic trays with water for about one hour.
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.

Use fine sand for germination-testing. Quarry or river sand is better than shore sand. If shore sand must be used, wash it thoroughly to remove all salts. Clean the sand by pasteurising it (at 180°F or 82°C and 5 psi in three cycles of one hour each) before use.

Figure 5.3 shows the stages in testing germination using sand.

Agar method

Agar is an alternative substrate to paper, particularly for testing germination in small and medium-sized seeds. Agar dissolves slowly in hot water and forms a viscous solution, which forms a stiff jelly upon cooling.

1. Sterilize the surface of the containers by wiping them with 70–95% alcohol or soaking in 20% bleach or hot water at 55°C for 10–15 minutes.
2. Label 9-cm Petri dishes and their covers (for small seeds), or any other heat-resistant germination test containers, with accession number, number of replicate and testing date.
3. Prepare 1% agar solution (WA) by dissolving 1 g of agar powder in 100 ml of warm distilled water heated on a hot plate.
4. Allow the solution to boil until the agar is completely dissolved, then cool slightly to 50°C and pour into the labelled Petri dishes or the other containers. The thickness of the substrate should be twice the thickness of the seeds.

Agar stays moist for up to one month and is particularly suited as a substrate in dormancy studies. It is susceptible to contamination from airborne fungi and bacteria, however, and sterile conditions are required in the laboratory when using this substrate.

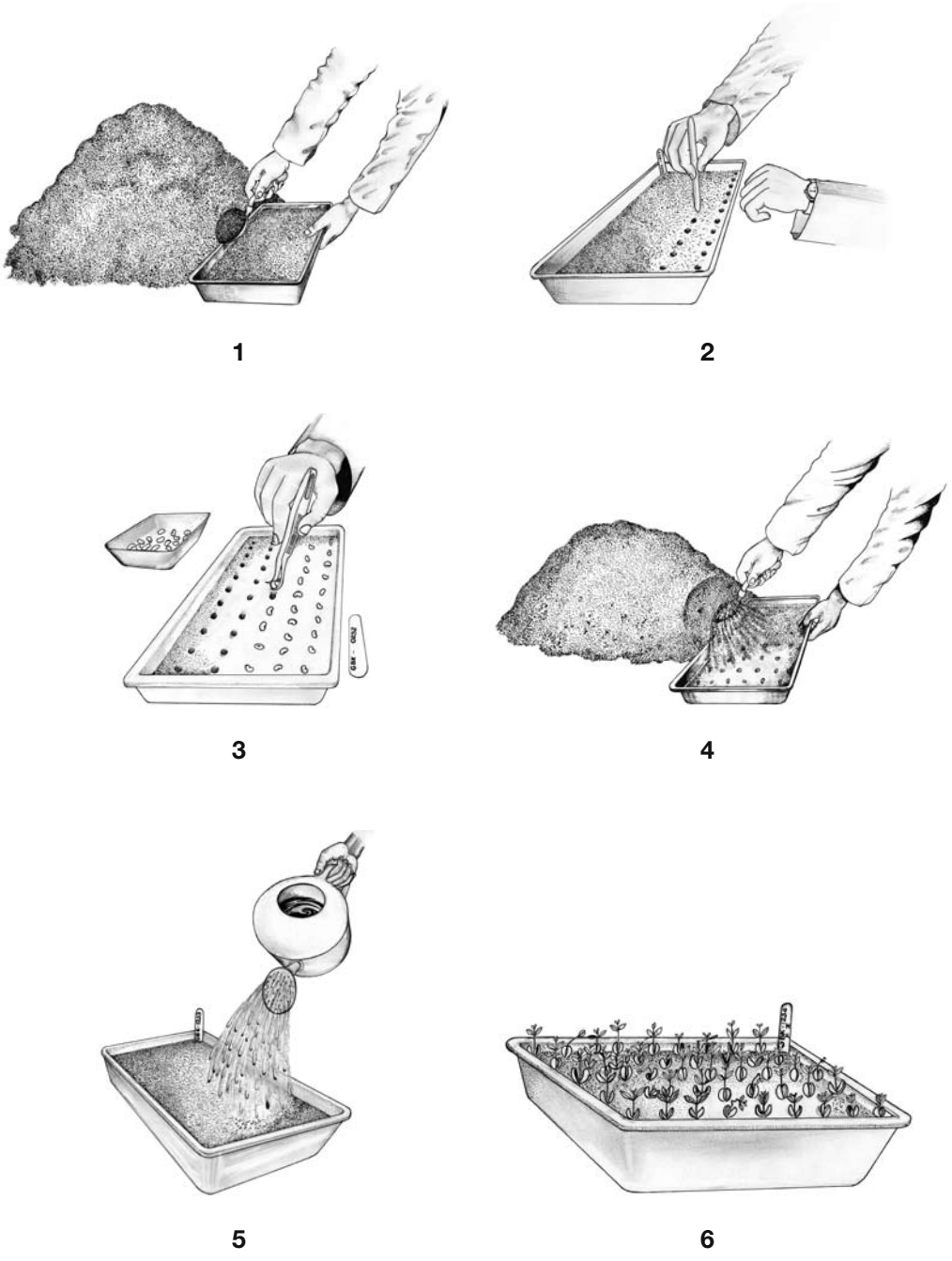

Figure 5.3. Seed germination testing in sand.

5. Arrange the seeds equidistantly on the surface of the agar.
6. Cover the dishes with their lids and place them in an incubator maintained at the recommended temperature for the species (see Table 5.1).
7. Run the test for the recommended period (see Table 5.1) and count the number of seeds that have germinated.

Step 3: Evaluation of germination tests

1. Seedlings removed during the course of germination testing are classified either as normal seedlings or abnormal seedlings (see Box 5.2).
   - Normal seedlings possess adequate root and shoot structures, which are essential for further development into plants.
   - Abnormal seedlings are incapable of further development and suffer deficiency, decay or weakness in their root or shoot systems.
2. It is important that germination tests are observed regularly and that normal seedlings and abnormal germinants are removed in order to allow other seedlings to develop in a less-crowded environment; it is also important to remove fungi-infected seeds in order to prevent the spread of infection. It is desirable to conduct an initial germination count after three or seven days, followed by a final count after seven or 14 days, depending on the species. Some species such as grasses require a test period of up to 21 or 28 days, and it is desirable to conduct an interim count at 14 days. Detailed germination procedures and periods for counting seedlings are provided in the ISTA and AOSA rules for seed-testing (see ISTA, 2005 and AOSA, 2005).
3. Record observations on the data sheet provided in Table 5.2.
4. Also record any abnormal seedlings or dead seeds removed during the first or interim counts (see Table 5.2)—they provide an indication of the progress of seed deterioration if a review is required at a later date.

Only those seedlings that are normal (those that demonstrate a capacity for sustained development under suitable conditions) are considered to have germinated. Abnormal seedlings should not be considered to have germinated.

Box 5.2. Seedling defects classified as abnormal (for more details, refer to ISTA [2003, 2005] or AOSA [2005]).

Roots

- Primary root stunted, stubby, missing, broken, split from the tip, spindly, trapped in the seed coat, with negative geotropism, glassy, decayed due to primary infection or with less than two secondary roots in monocots

Shoot (hypocotyl, epicotyl and mesocotyl)

- Short and thick, split right through, missing, constricted, twisted, glassy or decayed due to primary infection

Terminal bud/leaves

- Deformed, damaged, missing or decayed as a result of primary infection

Cotyledons

- Swollen, deformed, necrotic, glassy, separated or missing, and decayed due to primary infection

Examples of normal and abnormal seedlings in pea, groundnut, wheat and onion are shown as Figures 5.4 to 5.7.

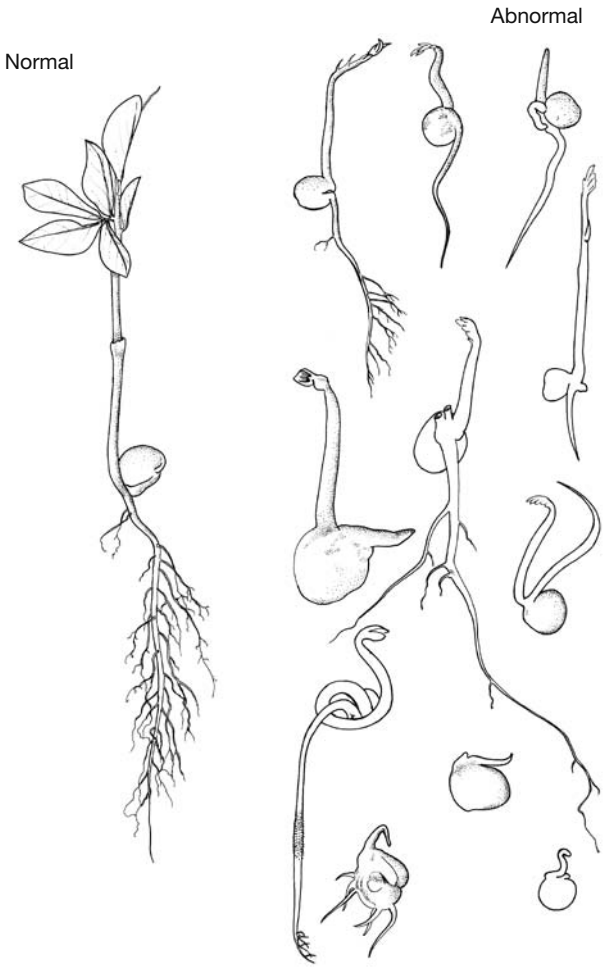

Figure 5.4. Seedling abnormalities in pea.

5. Upon completion of the germination test, count and record all un-germinated and dead seeds in each replicate.
6. Calculate the mean percentage germination of the accession from the results of all the replicates to determine the number of *normal seedlings* produced.
7. Repeat the germination test if the difference between the two replicates exceeds 10% or the maximum tolerance exceeds 2.5% probability (see Ellis et al., 1985).
8. 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.

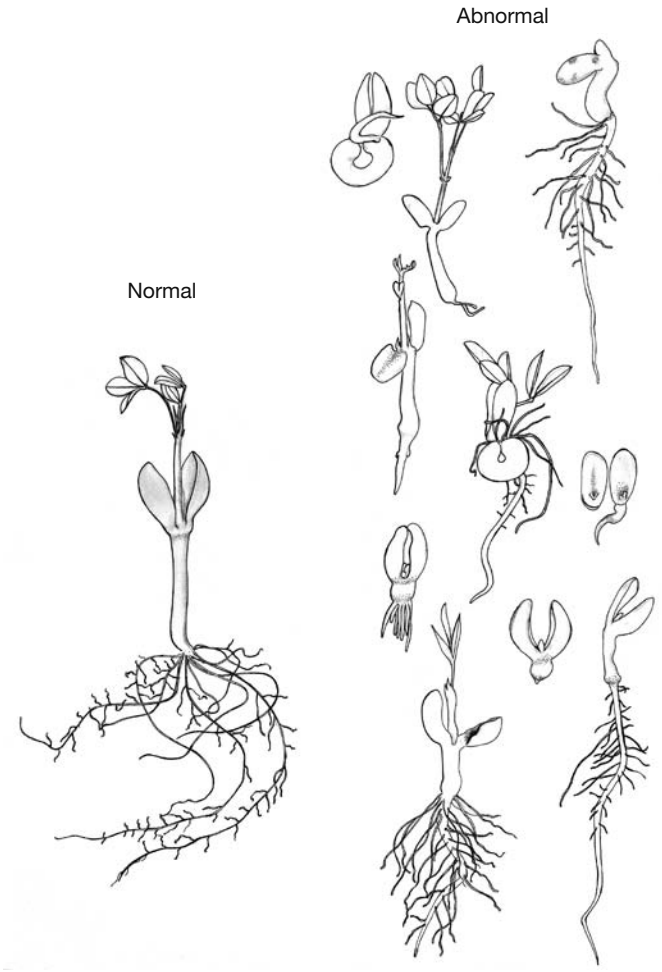

Figure 5.5. Seedling abnormalities in groundnut.

Why do some seeds fail to germinate?

Seeds fail to germinate either because they are dead or dormant. Dead seeds usually soften and rot during testing. 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. Un-germinated seeds found to have firm embryos are potentially viable. A high percentage of these seeds indicates that germination conditions were not optimal or that seeds are dormant.

Dormancy

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

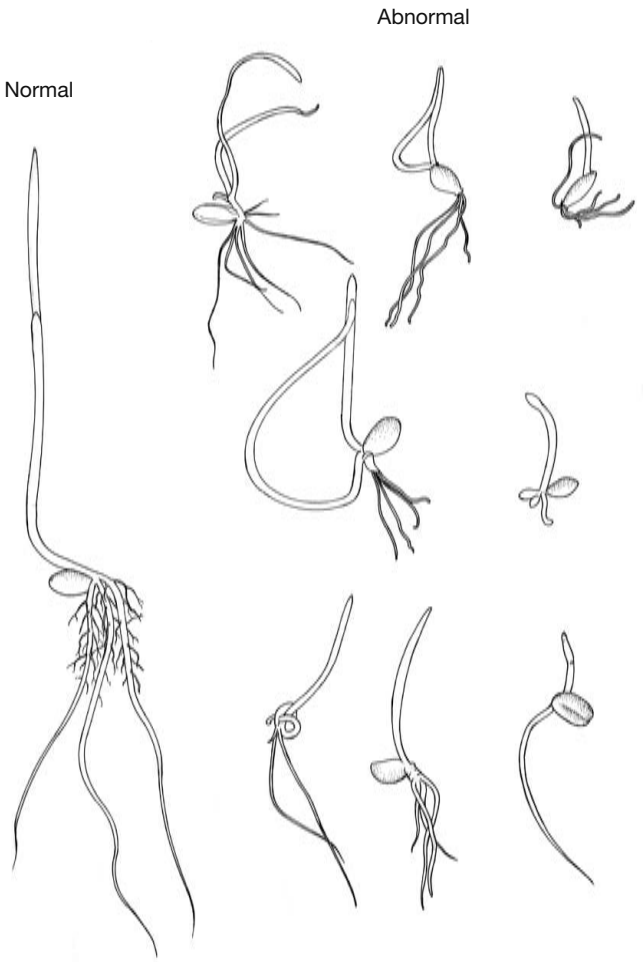

Figure 5.6. Seedling abnormalities in wheat.

How to determine if seeds are dormant

Seeds that remain hard, or absorb water but remain firm and in good condition, during germination tests are probably dormant. Seed dormancy is common in freshly harvested seeds and in many wild species of crop plants.

Types of dormancy

Seed-coat dormancy

Physical, chemical or mechanical conditions prevent uptake of moisture. Examples of seed-coat dormancy can be found in the

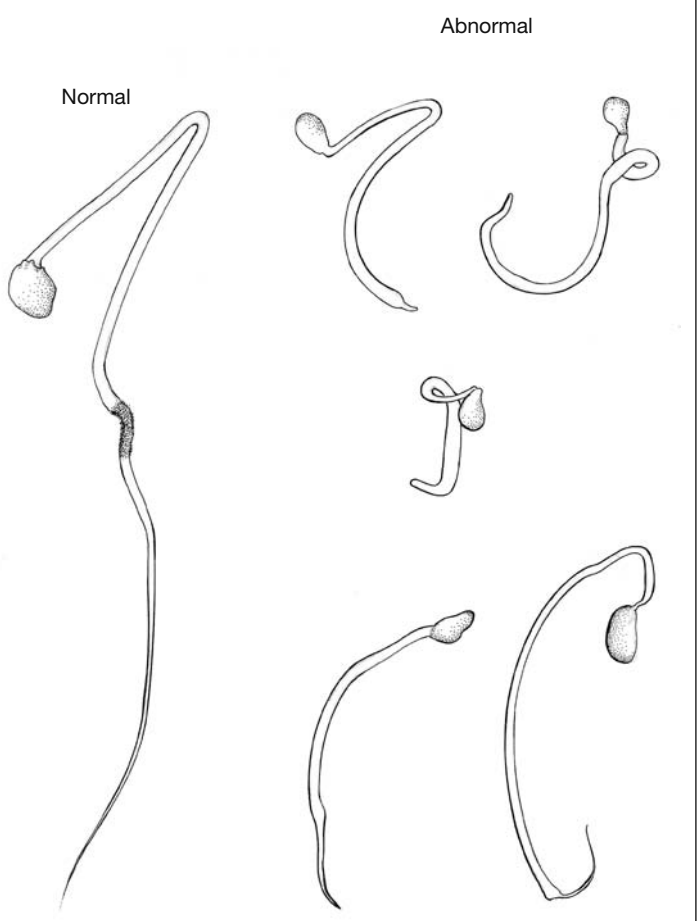

Figure 5.7. Seedling abnormalities in onion.

Anacardiaceae, Burseraceae, Cistaceae, Fabaceae, Geraniaceae, Malvaceae, and Rhamnaceae families.

Embryo dormancy

Inhibiting substances usually within the embryo or surrounding tissues prevent germination. Examples of embryo dormancy can be found in the Apiaceae, Iridaceae, Liliaceae, Papaveraceae and Ranunculaceae families.

In certain species, seed embryos are underdeveloped or not fully formed at seed dispersal. In these species, the embryo continues to

Table 5.2. Model data sheet to record germination results.

Crop/species: Substrate:
Accession number: Temperature:
Batch reference number: Light:
Date of storage: Special treatments:
Date of testing: Incubation time:

Replication		Normal seedlings				Total	Remarks
		I	II	III	IV		
No. of seeds tested						**Total**	**Remarks**
Date	**Days**						
Total germinated							
Abnormal							
Hard/dormant							
Dead							
Germination (%)							

grow after dispersal, and germination is prevented until the embryo reaches a species-specific critical length. Examples can be found in the Annonaceae, Apiaceae, Orchidaceae, Orobachaceae and Ranunculaceae families.

Dormancy can be also caused by a combination of impermeable seed or fruit coats and physiologically dormant embryos. For germination to occur, both types of dormancy must be broken. The order in which each type of dormancy must be broken depends on the species. Examples are *Ceanothus* (Rhamnaceae), *Tilia* (Tiliaceae) and *Rhus* (Anacardiaceae).

How to determine the type of dormancy

If removal of the seed coat does not result in germination, the dormancy mechanism is located in the embryo itself.

Dormancy-breaking treatments

In some seeds that are dormant at harvest, dormancy breaks down naturally over time. Other species require some form of pre-treatment. There are several methods used for specific genera.

Breaking seed-coat dormancy

Puncturing or scarifying the seed coat by piercing, nicking, chipping or filing with a knife, needle or sandpaper are preferred procedures to overcome seed-coat dormancy.

- Manual scarification is effective at any point on the seed coat, but the micropylar region should be avoided as it is the most sensitive part of the seed where the radicle is located (see Figure 5.8).

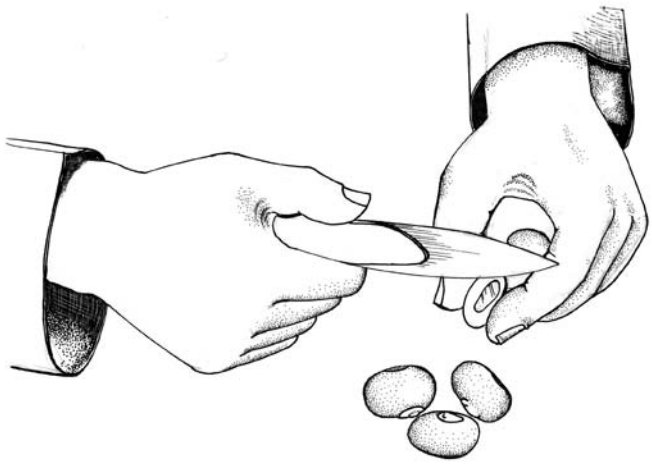

Figure 5.8. Manual scarfication of seed coat.

- If seed-covering structures prevent growth of the embryo, remove them to allow germination.
- If the seed coat contains inhibitors that prevent or delay germination, they can be leached out by placing the seed under running water for several hours or soaking the seed in a large volume of water that is changed every six to 12 hours.
- ISTA also recommends using concentrated sulphuric acid for 2–45 minutes depending on the species to scarify the seed coat. This method is expensive and dangerous, however, and should be followed with caution.
- To remove the waxy covering and allow imbibition, place the seeds in water at 75°C for three to six minutes. Care must be taken not to use high temperatures for long periods or boil the seeds.

Breaking embryo dormancy

There are several recommended treatments to overcome embryo dormancy (see Table 5.1). These include pre-chilling (also called cold stratification) for temperate and high-altitude species from the tropics;

preheating; application of gibberellic acid (GA_3) at low concentrations; addition of potassium nitrate (KNO_3) to the substrate; and light.

Pre-chilling (cold stratification)
Seeds are placed in containers on a moistened germination substrate and kept at 3° to 5°C in a refrigerator for seven days. For more dormant seeds, the treatment may be extended to 14 days. Once the stratification is complete, the containers are removed to incubators and seeds are allowed to germinate in recommended conditions.

Preheating
Seeds are treated at a temperature not exceeding 40°C for up to seven days with free air circulation before germination in recommended conditions.

Gibberellic acid
Germination test paper is moistened with a 0.05% solution of gibberellic acid (GA_3), prepared by dissolving 500 mg of GA_3 in 1 l water. Germination is then continued in recommended conditions.

Potassium nitrate
A 0.2% solution of potassium nitrate (KNO_3)—prepared by dissolving 2 g KNO_3 in 1 l water—is used to moisten the germination paper at the beginning of the test. Germination is continued in recommended conditions.

Light
Light may or may not be required for germination, depending on the species. When using constant temperatures for germination of species where light is required, the tests should be illuminated for at least eight hours of every 24-hour cycle. When alternating temperatures are used, any necessary application of light should coincide with the high-temperature cycle. Light intensity should be 750–1250 lux from cool, white lamps.

Many of the methods described above are specific to genera. Recommended dormancy-breaking treatments for common crops are given in Table 5.1. For information on other species, refer to Ellis et al. (1985).

Algorithm for developing suitable germination test procedures for species for which no information is available

Step 1

- Determine if seed coats are impermeable by checking imbibition of seeds placed on moist filter paper overnight. If seeds have

not imbibed water, scarify the seed coats using a scalpel blade and observe again after 12 hours. Proceed to germination when seeds have imbibed the water.

Step 2

- If the first step does not result in full germination and if the accessions are of temperate origin, test at constant temperatures of 15°C and 20°C. For accessions of tropical origin, use constant temperatures of 20°C and 25°C.
- If the accession origin is unknown or doubtful, test at 15°, 20° and 25°C.
- In all cases, apply light for 12 hours per day.

Step 3

- If the second step has not resulted in full germination, test a further sample of seeds in alternating temperatures 25°/10°C (12 hours and 12 hours) for accessions of temperate origin and 35°/20°C (12hours and 12hours) for accessions of tropical origin.
- If light is applied for 12 hours per day, it should coincide with the upper temperature cycle.
- If the accession's origin is unknown or doubtful, test a sample of seeds at each temperature.

Step 4

- If the third step has not resulted in full germination, add 0.1–0.2% potassium nitrate (KNO_3) to the test substrate in the most successful temperature regime determined in steps 2 and 3.

Step 5

- If the fourth step has not resulted in full germination, pre-chill the seeds at 2°C to 6°C for eight weeks and test for germination in the most successful regime determined in steps two through four.

Step 6

- If full germination is not obtained, estimate viability using the tetrazolium test described below. The results of this test will indicate if the failure to achieve full germination is due to the presence of dead seeds.
- If the tetrazolium test indicates that dormancy is not broken and seeds are viable, try other dormancy-breaking treatments such as gibberellic acid (GA_3) or pre-heating at 40°C for three to seven days.

Tetrazolium test for seed viability

The tetrazolium test can be used as a backup procedure to identify viable but dormant seeds that have failed to germinate at the end of a germination test. The procedure for this test is indicated below.

The tetrazolium test is not an absolute test of seed viability. To gain reliability, the test must be compared with the results of germination tests for each species.

Preconditioning

1. Remove the seed-covering structures (glumes, etc.).
2. Precondition the seeds by soaking in water or by placing them in a moist medium at 30°C. No preconditioning is necessary when un-germinated seeds are evaluated at the end of a germination test.

Preparing tetrazolium chloride solution

The tetrazolium solution should be between pH 6 and 8 to achieve best results. To prepare 1 litre of buffered 1% tetrazolium chloride solution:

1. Dissolve 3.631 g of potassium dihydrogen phosphate (KH_2PO_4) in 400 ml of distilled water.
2. Dissolve 7.126 g of disodium hydrogen phosphate ($Na_2HPO_4.2H_2O$) in 600 ml of distilled water.
3. Mix the two solutions to prepare the buffer.
4. Dissolve 10 g of 2,3,5,-triphenyl tetrazolium chloride in the 1 litre of buffer solution.

To produce 0.5% tetrazolium solution, mix one part stock solution with one part distilled water. Tetrazolium chloride solution should be stored in dark and cold conditions for short periods.

Staining

1. Bisect the seeds longitudinally through the embryo with a razor blade.
2. Discard half of each seed and place the other half in the staining solution at the recommended concentration (see Table 5.3) in a glass vial.
3. Place the vials in an incubator in a dark area at the recommended temperature and duration for each species (see Table 5.3).
4. After staining, wash the seeds several times in distilled water to remove excess stain.
5. Immerse the seeds in lactophenol solution (1 litre of lactophenol prepared from 200 ml phenol, 200 ml lactic acid, 400 ml glycerine, and 200 ml water) for one to two hours before evaluating the seeds.
6. Evaluate the seeds for a staining pattern under a low-powered binocular microscope; viable tissues stain bright red. Pink and very dark red stains indicate dead tissue.
7. Classify the seeds into three categories depending on staining pattern:
   - completely stained seeds that are viable;
   - completely unstained seeds that are nonviable; and
   - partially stained seeds that will produce either normal or

Table 5.3. Concentration, temperatures and period of staining with tetrazolium solution (for Annex I crops of the International Treaty on PGRFA).

Crop	Species	Preconditioning	Staining
Barley	*Hordeum vulgare*	Imbibe or soak, 6–18h	0.5%, 3h, 30°C
Beans	*Phaseolus* spp.	Imbibe 18–24h, then soak, 2–3h	0.5–1%, 6–24h, 30°C
Brassica	*Brassica* spp.	Imbibe or soak, 16–18h	0.5–1%, 3–6h, 30°C
Chickpea	*Cicer arietinum*	Imbibe or soak, 18h	1%, 6–24h, 30°C
Cowpea	*Vigna unguiculata*	Soak, 22h	0.5–1%, 16–24h, 30°C
Eggplant	*Solanum melongena*	Imbibe or soak, 18h	0.5–1%, 6–24h, 30°C
Faba bean	*Vicia faba*	Soak, 22h	0.5–1%, 16–24h, 30°C
Finger millet	*Eleusine corocana*	Soak, 18h, 5°C	0.5%, 3h, 30°C
Lentil	*Lens culinaris*	Imbibe, 18h, then soak, 2–3h	1%, 6–24h, 30°C
Maize	*Zea mays*	Imbibe or soak, 18h	0.5–1%, 2–6h, 30°C
Pea	*Pisum sativum*	Imbibe 18–24h, then soak, 2–3h	0.5–1%, 6–24h, 30°C
Pearl millet	*Pennisetum glaucum*	Imbibe or soak, 6–18h	0.5–1%, 6–24h, 30°C
Rice	*Oryza sativa*	Imbibe or soak, 18h	0.5%, 3h, 30°C
Rye	*Secale cereale*	Imbibe or soak, 6–18h	0.5%, 2–3h, 30°C
Sorghum	*Sorghum bicolor*	Imbibe, 16h, 30°C	0.5–1%, 0.5–1h, 40°C
Sugar beet	*Beta vulgaris*	Imbibe or soak, 16–18h	1%, 24–48h, 30°C
Sunflower	*Helianthus annuus*	Imbibe or soak, 18h	0.5–1%, 3–6, 30°C
Triticale	*Triticosecale*	Imbibe or soak, 6–18h	0.5%, 2–4h, 30°C
Wheat	*Triticum aestivum*	Imbibe or soak, 6–18h	0.5%, 2–4h, 30°C

abnormal seedlings, depending on the intensity and pattern of staining (see ISTA 2005 for more information).

Figures 5.9 and 5.10 show patterns of tetrazolium staining in dicot and monocot seeds, respectively.

Documentation

Documenting viability data is crucial for the efficient management of germplasm collections as it enables genebank staff to make informed decisions regarding the timely regeneration of material (see Chapter 8). Suggested descriptors to document accession-level information on viability (germination) testing include the following:

- 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

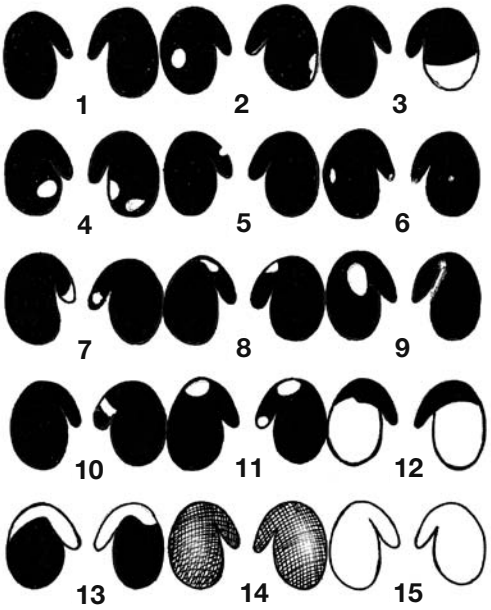

Figure 5.9. Staining pattern after tetrazolium test in dicot seeds. Illustrations depict both sides of seeds. Numbers 1–6 are germinable seeds and numbers 7–15 are nongerminable seeds (adapted from AOSA, 2005).

- 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

Further reading

Association of Official Seed Analysts. 2005. Rules for testing seeds. Association of Official Seed Analysts, USA.

Baskin, C. C. and Baskin, J. M. (1998) Seeds: Ecology, Biogeography and Evolution of Dormancy and Germination. Academic Press, San Diego, USA.

Ellis, R.H., Hong, T.D and Roberts, E.H. 1985. Handbook of seed technology for genebanks. Volume 1: Principles and methodology. Handbooks for Genebanks. No. 2. IBPGR, Rome, Italy.

FAO/IPGRI, 1994. Genebank standards. FAO and IPGRI, Rome, Italy.

ISTA. 2003. ISTA Handbook for Seedling Evaluation. International Seed Testing Association. Bassersdorf, Switzerland.

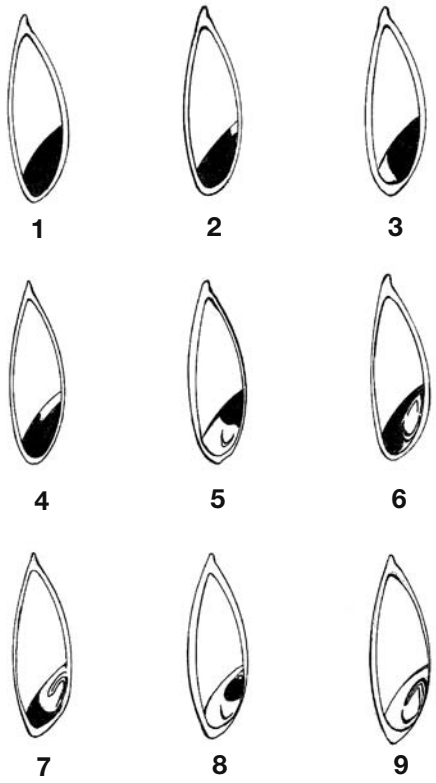

Figure 5.10. Staining pattern after tetrazolium test in monocot seeds. Numbers 1–4 are germinable seeds and numbers 5–9 are nongerminable seeds (adapted from AOSA, 2005).

ISTA. 2005. International Rules for Seed Testing. Edition 2005. International Seed Testing Association, Bassersdorf, Switzerland.

Smith, R.D., Dickie, J.B., Linington, S.H., Pritchard, H.W and Probert, J.R. (eds.). 2003. Seed conservation: Turning science into practice. Royal Botanic Gardens, Kew, UK.

5.2 Seed health testing

What is seed health?

Seed health refers to the disease status of a seed sample and the presence or absence of disease-causing organisms and pests.

What is seed health testing?

Seed health tests determine the status of a seed sample, seed lot or accession with regard to diseases affecting that crop or wild species.

Why is seed health testing important?

Crops are frequently infected with a range of common seed-borne pathogens that may not be visible or easily recognized during seed collection. Seed-borne inoculums reduce storage longevity and cause poor germination or field establishment. Seed-borne inoculums also promote disease in the field, reducing the value of crops. Exchange of infected seeds may allow spread of diseases and pests into new regions. Genebanks should ensure that seeds prepared for conservation are free from seed-borne diseases and pests.

Common seed-borne pests and pathogens

There are four main types of common organisms that are carried in seeds and affect a wide range of crops:

- Fungi
- Bacteria
- Viruses
- Insects

Specific methods for detecting pathogens vary by organism and host, and specific methods are required for accurate identification of most pathogens.

Methods of detecting pests and pathogens

Seed-health standard

Examine a representative sample of seeds for the presence of pathogens using one or more of the following methods. Usually, a sample of 400 seeds in replication of 100 seeds each is drawn for examination. Sample size can be decreased for small seed lots.
If the percentage of seeds infected is greater than 5%, the seed lot can be considered unsuitable for conservation.

Visual examination

The simplest method to detect diseases and pests is to examine dry seeds with the naked eye or under a low-powered microscope. This method reveals freely moving insects, eggs, mites, fungal fructifications like sclerotia, galls, smut balls, bacterial masses and infected plant debris. Examination of dry seeds under ultraviolet or near-ultraviolet light reveals infections of certain fungi and bacteria through emission of fluorescence.

Seedling evaluation

Seeds should be planted in sterilized soil in a screenhouse. Seedlings should be observed immediately after germination and any plants exhibiting virus-like symptoms such as leaf mottling, curling or yellowing should be sampled and tested for viruses (see

below). Seedlings infected with bacteria or fungi may die and should be examined further in a laboratory, and the samples should be plated for identification of the pathogen (see below).

If infection is suspected but no symptoms have been observed after the second true leaf has emerged, it may be necessary to carry out serological tests for latent or symptom-less infection by viruses. Most legume viruses will express conspicuous symptoms at the seedling stage.

Seed-washing technique

This is useful for testing surface-borne, contaminating fungi such as smuts, bunts, downy mildews, powdery mildews and rusts.

1. Place 2 g of the seed sample in a test tube, add 2 ml of sterile water and mix well for five to ten minutes.
2. Centrifuge the supernatant solution at 200 rpm for ten minutes and observe the sediments under a microscope for fungal structures.

Incubation methods

The blotter and agar-plate methods are simple and inexpensive ways to detect seed-borne fungi that respond to sporulation.

Blotter test

Blotter tests are similar to germination tests in that seeds are placed on moistened layers of absorbent paper and incubated under conditions that promote fungal growth.

1. Line the base of sterilized Petri dishes with three layers of absorbent paper moistened with sterile water.
2. Drain off excess water and place 20–25 seeds manually with forceps.
3. Evenly space the seeds to avoid contact.
4. Incubate the seeds under near-ultraviolet light in alternating cycles of 12 hours light/darkness for seven days at 20°±2°C.
5. Examine the Petri dishes under a stereo-binocular microscope for fungi developing on the seeds.

Profuse seedling growth may make interpretation difficult. This may be overcome by adding 2,4-D sodium salt to provide a 0.2% moistening solution.

Agar-plate method

This is the most common method used for identifying seed-borne fungi. Different fungi and even different strains of the same fungi require different media for growth and sporulation. Near-ultraviolet light with a wavelength 300–380 nm (also called black light) may be required. Simple media include a combination of vegetables, carbohydrate or sugar sources and agar, and can be made by

combining boiled and mashed vegetables with agar when commercial mixes are unavailable. The most commonly used media are potato dextrose/sucrose agar and oatmeal agar.

1. Prepare the medium by mixing 1 g potato dextrose agar powder in 100 ml distilled water.
2. Sterilize the mixture in an autoclave for 15–20 minutes and cool to 50°C.
3. Carefully pour the mixture into sterile Petri dishes, lifting the lid enough only to pour in the agar to avoid contamination.
4. Allow it to cool and solidify for 20 minutes.
5. Surface-disinfect the seeds by pre-treating them for one minute in a 1% sodium hypochlorite (NaOCl) solution prepared by diluting 20 parts domestic bleach (5% NaOCl) with 80 parts water.
6. Place approximately ten seeds (depending on size) on the agar surface with forceps.
7. Incubate the Petri dishes at 20°–25°C for five to eight days.
8. Identify the seed-borne pathogens on the basis of colony and spore characteristics.

Sometimes bacterial colonies develop on the agar and inhibit fungal growth, making identification difficult. This can be overcome by adding an antibiotic such as streptomycin (500 ppm) to the autoclaved agar medium after it cools to 50°–55°C.

Polymerase chain reaction (PCR) method

PCR is an *in vitro* technique to amplify a small quantity of a specific nucleotide sequence exponentially in the presence of a template sequence with two oligonucleotide primers that hybridize to opposite strands and flank the region of interest in the target DNA. The reaction is cycled, involving template denaturation, primer annealing, and extension of the annealed primers by DNA polymerase until enough copies are made for further analysis. PCR can allow the detection of very small amounts of a pathogen in a sample by amplifying the pathogen sequences to a detectable level. PCR is especially useful to detect diseases because of its speed and accuracy, but it is an expensive technique—it can be used to detect any organism that has DNA by using positive and negative controls for comparison. Once the sequence of the organism is known, specific probes can be made to detect strains of pathogens.

Nucleic acid hybridization assays (called southern and northern blotting), in which DNA or RNA is transferred from an electrophoresis gel onto a membrane and then the nucleic acids are detected with a labelled probe, can also be used. The nucleic acid spot hybridization (NASH) technique, in which a labelled DNA pathogen hybridizes directly to the pathogen DNA immobilized on a nylon membrane, can also be used

without going through the PCR stage. These techniques are constantly being refined and new procedures are becoming available for specific pathogen detection. For more information, refer to Albrechtsen (2005).

Serological and other methods

Enzyme-linked immunosorbent assay (ELISA)

ELISA is a diagnostic method that uses proteins called antibodies to detect plant pathogens. This assay is based on the ability of an antibody to recognize and bind to a specific antigen—a substance associated with a plant pathogen. The antibodies used in diagnostics are highly purified proteins produced by injecting a warm-blooded animal (like a rabbit) with an antigen associated with a particular plant disease. The animal reacts to the antigen and produces antibodies, which recognize and react only with the proteins associated with the causal agent of that plant disease. Colour changes on the unit's surface indicate a positive reaction (disease present).

There are many different types of ELISAs that can detect the presence of protein. A detailed description of these is beyond the scope of this publication and genebank staff members are advised to refer to Albrechtsen (2005). However, the general procedures for two most common methods—antigen-coated plate (ACP-ELISA) and tissue-blot immunoassay (TBIA)—are given in Annex II. For more details, refer to Lin et al. (1990).

Indicator plant method

This is especially useful for detecting bacteria and viruses. Seed extracts are prepared and inoculated on indicator plants like tobacco. The pathogens are identified based on the symptoms that develop. Indicator plants can also be used to separate different viruses by virus-host specificity.

Documentation

Suggested descriptors to document accession-level information on seed health-testing include the following:

- Source of the material for testing
- Type of material (leaf, stem, root, seeds)
- Number of plants sampled and tested per replicate
- Number of replicates
- Organisms tested for
- Method of testing
- Date of test
- Duration of test, if appropriate
- Diseases identified
- Incidence of each disease (%)

Further reading

Albrechtsen, S.E. 2005. Testing methods for seed-transmitted viruses: Principles and protocols. Oxford University Press, Oxford, UK.

ISTA. 2005a. International Rules for Seed Testing. Edition 2005. International Seed Testing Association, Bassersdorf, Switzerland.

Lin, N.S., Hsu, Y.H. and Hsu, H.T. 1990. Immunological detection of plant viruses and mycoplasm-like organisms by direct-tissue blotting in nitrocellulose membranes. Phytopathology, 80: 824–828.

5.3 Seed testing for inadvertent introduction of transgenes

What are transgenes?

Transgenes are genes that are introduced into another organism or species through recombinant DNA techniques. Transgenic plants carry transgenes in their genomes and transmit them to their progeny through normal reproduction.

Why determine the presence of a gene/transgene?

One of the most important components of proper genebank management is testing for the presence of a gene or phenotype. This is critical for various phytosanitary requirements, but is also becoming important for the detection of transgenes. There are a number of reasons why it is important to detect the presence of a gene/transgene in a genebank accession. While not an exhaustive list, these include:

- regulatory issues, especially related to phytosanitary or biosafety, where the country of import, and potentially the country of export, requires reporting on the presence of such genes;
- situations in which the presence of such a gene/transgene could affect intellectual property rights either in the country where the genebank is located or in a country where the accession is to be sent; and
- social issues necessitating that genetic identity be stated or that certain genes/transgenes should be limited.

When should one detect the presence of a gene/transgene?

It is generally accepted as unwise for crops containing transgenes to be incorporated into germplasm collections. The risk of inadvertent inclusion of transgenes can be classified as follows:

- High probability: typically out-crossing crops with sexually compatible relatives on which extensive research in the field or commercial release is ongoing.
- Low probability: typically crops which are highly self-pollinating, multiplied vegetatively or crops for which genetic engineering has either not been done or is in its very early stages.

- Medium probability: the remainder of crops.
- Immediate attention: crops with transgenes that are already commercially distributed.
- Near-future attention: experimental field work is ongoing or expected within one to three years.
- Long-term attention: crops for which no significant work has been done in the field.

Genebanks should take proactive steps to limit the risk of exotic genes, including transgenes, in their *ex situ* collections. Accessions that do not require testing include:

- species where no transgenic events (commercial or research) have occurred;
- accessions for which there were no commercial transgenes present at the time of acquisition (such as maize prior to 1996) or no transgenics near the collecting site; and
- accessions for which there have been transgenic events, but good management practices have been followed in the accession process.

In 2004, the Genetic Resources Policy Committee (GRPC) and the Science Council of the CGIAR organized a technical workshop to explore the ways and means to handle unintentional presence of transgenes in germplasm collections, with the goal of providing technical inputs into a process that would enable CGIAR Centre genebanks to draw up procedures aimed at preventing the unintentional introgression of transgenes into the collection. Further to this workshop, a guiding principle was prepared and adopted by the GRPC. For more information on this subject consult the Policies and Ethics section of Bioversity's website (www.bioversityinternational.org/About_us/Policies_and_Ethics/index.asp; last visited 20 December 2006). These guiding principles were also considered at the Third Session of the Intergovernmental Technical Working group on Plant Genetic resources for Food and Agriculture held at FAO Rome October 26-28 October 2005. Further information about that meeting is available from the Commission web site http://www.fao.org/waicent/FaoInfo/Agricult/AGP/AGPS/pgr/ITWG3rd/docsp1.htm

Procedures to prevent unintentional gene flow from genetically modified organisms (GMOs)

Transgenes and conventional genes are subject to the biological processes of mutation, gene flow, introgression, recombination and natural selection. Therefore, best practices for preventing introgression of conventional genes also provide an appropriate basis for preventing introgression of transgenes.

Germplasm is most at risk from gene flow during regeneration (see Chapter 8) and controlling gene flow is essential to ensure genetic integrity. To reduce the risk in crops where transgenes are commonly part of new cultivars, it is recommended that regeneration be carried out in isolation from any areas where transgenic crops are likely to be grown.

Information on crops' transgenic status is essential to determine what measures, if any, are needed to confirm that germplasm is free of transgenes. It is recommended that:

- all results be made publicly available as soon as they have been confirmed;
- all procedures and supporting information be presented;
- the appropriate authority in the country of origin be informed in cases where transgenes are detected; and
- for commercially released genetically modified crops and crops in experimental development, genebanks maintain a database of crops and their status in transgenic research.

Once an accession has either been determined to not require testing or has tested negative, follow appropriate regeneration and maintenance procedures to maintain genetic integrity, as for all accessions.

Procedures for testing for presence of GMOs

The two basic methods to detect the presence of a gene/transgene are ELISA and PCR amplification. Both methods have already been described and are robust, although each has advantages and disadvantages. For example, ELISA detects the presence of a gene product (protein) and thus requires an expressing gene. Test kits are commercially available for most commercial events, which can be used in the field. On the other hand, PCR can detect non-expressing gene sequences, in almost all tissues, but it is more difficult to perform and therefore not practical in the field. In most cases, the detection of a positive result using one method should be confirmed with a second method. If the materials are being analyzed at the molecular level for fingerprinting or diversity studies, an additional test for the presence of a transgene can be performed at minimal cost.

The genes/transgenes that should be used in such tests include the current commercialized major events for the species. These can normally be found on the Internet and are indicated in the tests provided by commercial testing services (either as ELISA kits or PCR services). These will change as new transgenic events are introduced into the market or events become obsolete and are

removed, although the need to test may continue for some time. The number of seeds in any accession may limit the level of detection. More information and technical guidance on sampling and detection of GMOs can be found at www.europa.eu.int/comm/environment/biotechnology/pdf/recom2004_787.pdf.

An updated list of validated methods is also available at http://biotech.jrc.it.

Documentation

Suggested descriptors to document accession-level information on the presence of transgenes include the following:

- Source of the material for testing
- Type of material (leaf, seedling, seed)
- Number of plants sampled and tested per replicate
- Number of replicates
- Transgenes tested
- Method of testing
- Date of test
- Duration of test, if appropriate
- Transgenes identified
- Incidence of each transgene (%)