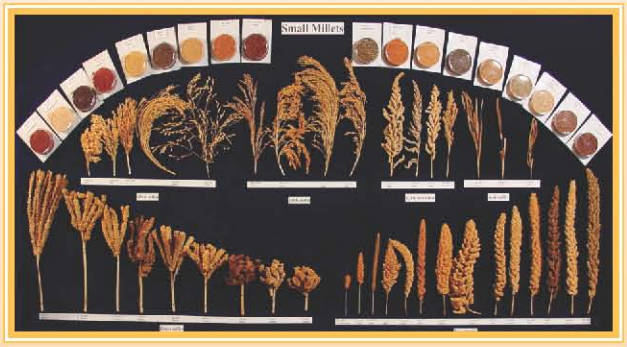
Technical Manual no. 10

Managing and Enhancing the Use of Germplasm – Strategies and Methodologies



ICRISAT
Science with a human face

International Crops Research Institute for the Semi-Arid Tropics

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Managing and Enhancing the Use of Germplasm – Strategies and Methodologies

Hari D Upadhyaya and CL Laxmipathi Gowda

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Patancheru 502 324, Andhra Pradesh, India
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4D. Seed viability testing

Viability tests measure how many seeds germinate and develop into plants, which reproduce themselves.

- Viability of accessions should be tested:
  - before seeds are packaged and placed in the genebank, and
  - at regular intervals during storage.

Many methods are available to test seed-viability. The most accurate method to test seed viability is the germination test using appropriate procedure.

4D.1. Germination test

Complete germination can be achieved only under optimum conditions of light, temperature and water. The requirements for germination vary with species as shown in Table.4D.1.1.

Table 4D.1.1. Recommended conditions for germinating seeds of ICRISAT mandate crops.

Crop	Substrate*	Temperature	Special requirements**
Sorghum	BP	20/30°C (16/8h); 20°C	0.2% KNO_3 for wild species
Pearl millet	TP	20/30°C (16/8h); 20°C	
Chickpea	BP	20°C	Mechanical scarification for wild species
Pigeonpea	BP	25°C	Mechanical scarification for wild species
Groundnut	BP	25°C	Remove shell, 0.2% ethrel***
Finger millet	TP	20/30°C (16/8h)	
Foxtail millet	TP	20/30°C (16/8h)	
Little millet	TP	Not available	
Proso millet	TP	20/30°C (16/8h)	Light 180 × 10^{-6} m^{-2} s^{-1}, 12 h/d
Barnyard millet	TP	20/30°C (16/8h)	Prechill, light
Kodo millet	TP	20/30°C (16/8h)	

* TP = Top of Paper, BP = Between Paper (Paper towel method)

** Freshly harvested seeds and wild species of most crops show dormancy, ie, the seeds remain hard and firm during the germination test. Special treatments are required to overcome dormancy.

*** Prepared by diluting 2 mL ethrel (2-chloroethylphosphonic acid) with 998 ml distilled water.

Sample size

- Use a minimum of two replicates each of 50 or 100 seeds for testing initial germination and two replicates each of 25 or 50 seeds for subsequent tests, depending on available seed quantity.
- Take a random sample of seeds from the container.
- If the seeds are very dry (moisture content <8%) expose them to ambient atmosphere for 24 h to raise the moisture content before testing for germination.

Two methods are used for testing germination:

A. Top of paper method for millets.

B. Between paper (Rolled towel) method for sorghum, chickpea, pigeonpea and groundnut.

Paper towel is used as substrate for germination in both these methods.

Quality of paper towel

- The paper used as substrate should not be toxic to developing seedlings.
- It should be able to absorb and supply sufficient moisture to the seeds to germinate.
- It should be strong enough not to fall apart when handled and not to be penetrated by the roots of developing seedlings.

All new batches of paper towel substrate should be tested for their quality on receipt.

Simple test for paper quality

Presence of toxic substances

- Cut the paper to size and place in a 9-cm petri dish.
- Moisten the paper with sufficient water.
- Test the seeds of sensitive species like Bermuda grass (*Cynodon dactylon*), if available, or finger millet (*Eleucine coracana*) for germination on the moistened paper:
- Evaluate the root development after 5 days.
  - ➢ classic symptoms of paper toxicity are shortened and discolored root tips.

Paper strength

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

Moisture absorption

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

4D.1.1. Top of paper method

Seeds are germinated on top of moist paper (Whatman Grade 181) in a petri dish (Fig. 4D.1.1.1-4).

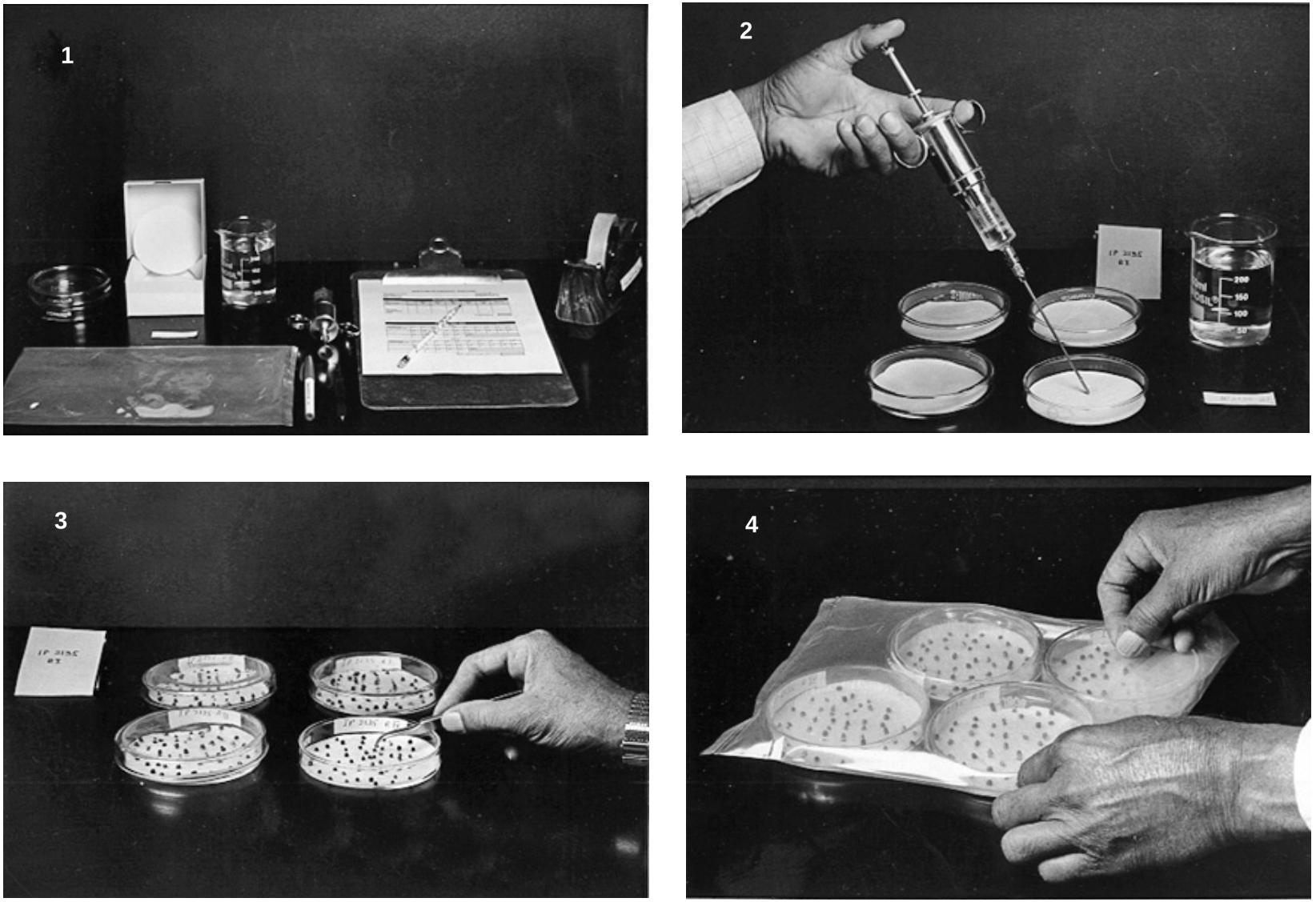

Figure 4D.1.1.1-4. Testing germination of seeds on the top of filter paper.

- Place the paper in 9-cm petri dishes.
- Moisten it with about 4 ml of distilled water.
- Put a label in the petri dish with accession number, number of replicate and date of the test.
- Spread the seeds at regular distance on the surface of the paper.
- Cover the petri dishes and keep them in a plastic bag to prevent drying.
- Place the petri dishes in an incubator maintained at the recommended optimum temperature.

4D.1.2. Between Paper (Paper Towel) method

Seeds are germinated between two layers of moist paper towels (Fig. 4D.1.2.1-7).

- Cut the paper to a convenient size to hold one replicate of the seeds (Fig. 4D.1.2.1.1).
- Label the paper on the outside at one end with the accession number, replicate number and the date of testing (Fig. 4D.1.2.1.2).
- Moisten the paper towels with water.
- Arrange the seeds in rows at regular intervals 4 cm from the top edge, leaving 3–4 cm gap on the sides (Fig. 4D.1.2.1.3).

Scarify (puncture the seed coat with a razor blade or scalpel without damaging the embryo) the seeds of Cicer and Cajanus species before sowing.

- Cover the seeds with another sheet of dry paper towel (Fig. 4D.1.2.1.4).
- Roll the paper loosely from the label end (Fig. 4D.1.2.1.5).
- Put a paper clip to hold the rolled paper towels from falling apart (Fig. 4D.1.2.1.6).
- Keep the rolls in a plastic tray (Fig. 4D.1.2.1.7).
- Add sufficient quantity of distilled water (covering the bottom 3-cm of rolls) to the tray.
- Place the tray in an incubator maintained at recommended temperature (Fig. 4D.1.2.2) (see Table 4D.1.1).

Fungal contamination is common when testing germination of legume seeds. Adopt the following laboratory practices to minimize infection:

- Use proper spacing of seeds — increase the distance between seeds and use greater number of replicates.
- Provide optimum environment for germination — temperature regime should be suitable and the test environment must be well aerated.
- Ensure cleanliness of germination test media and containers — making sure that these are not sources of inoculum.
- Avoid imbibition injury (by prior humidification of the seeds) that could lead to leakage of cell contents and provide source of nutrients to fungi.
- Promptly remove decaying seeds to prevent the spread of fungi to neighboring seeds.


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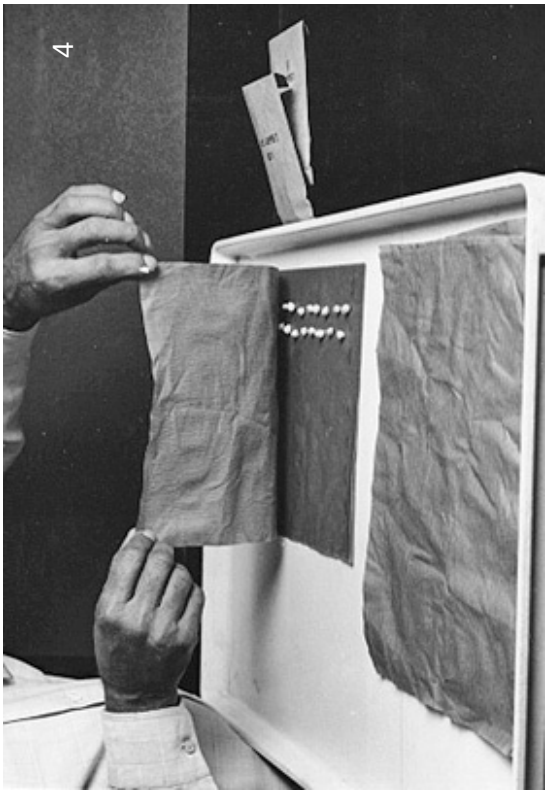
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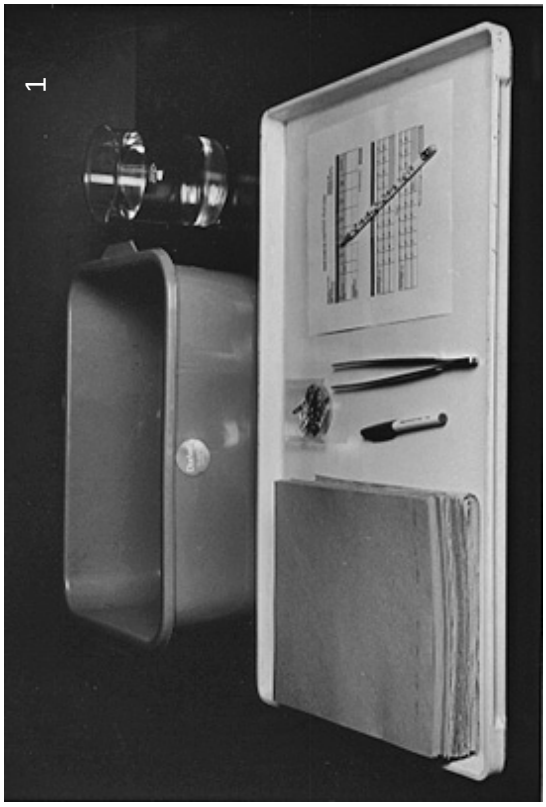
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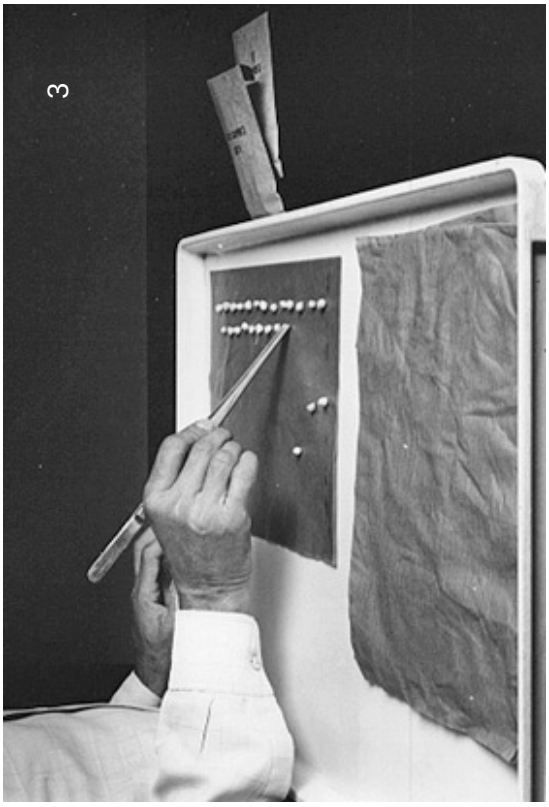
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Figure 4D.1.2.1-7. Testing germination of seeds between moist paper towels.

- Remove seed covering structures before tests when these are found to be sources of infection.
- Remove sprouted seeds (seeds that germinated before harvest and subsequently dried), which can be a source of severe infection.
- Treat seed with Thiram (tetramethyl thioperoxy dicarbonic diamide).

4D.1.3. Evaluation of germination tests

- Evaluate the seedlings 7 days after sowing.
- Scarify the hard and ungerminated seeds of chickpea and pigeonpea and evaluate at 14 days after sowing.
- Classify the seedlings removed during course of germination test as normal seedlings and abnormal seedlings.
  - ***normal seedlings are capable of developing into plants given favorable conditions and possess adequate root and shoot structures,***
  - ***abnormal seedlings are those incapable of further development and suffer deficiency, decay or weakness in their root or shoot system.***

Seedlings with the following defects are classified as abnormal (see Figs. 4D.1.3.3 and 4):

- ***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, and with less than two secondary roots.
- ***Shoot*** (hypocotyl, epicotyl and mesocotyl)
  - short and thick, split right through, missing, constricted, twisted, glassy, and decayed due to primary infection.
- ***Terminal bud/leaves***
  - deformed, damaged, missing, and decayed due to primary infection
- ***Cotyledons***
  - swollen, deformed, necrotic, glassy, separated or missing, and decayed due to primary infection
- Record observations in the data sheet shown as Annexure 4D.1.
- Update the inventory database with information from germination test.
- Repeat the germination test if the difference between the two replicates exceeds the maximum tolerance limits at 2.5% probability (Appendix 1).

Figure 4D.1.2.2. Trays containing rolled paper towels placed in an incubator.

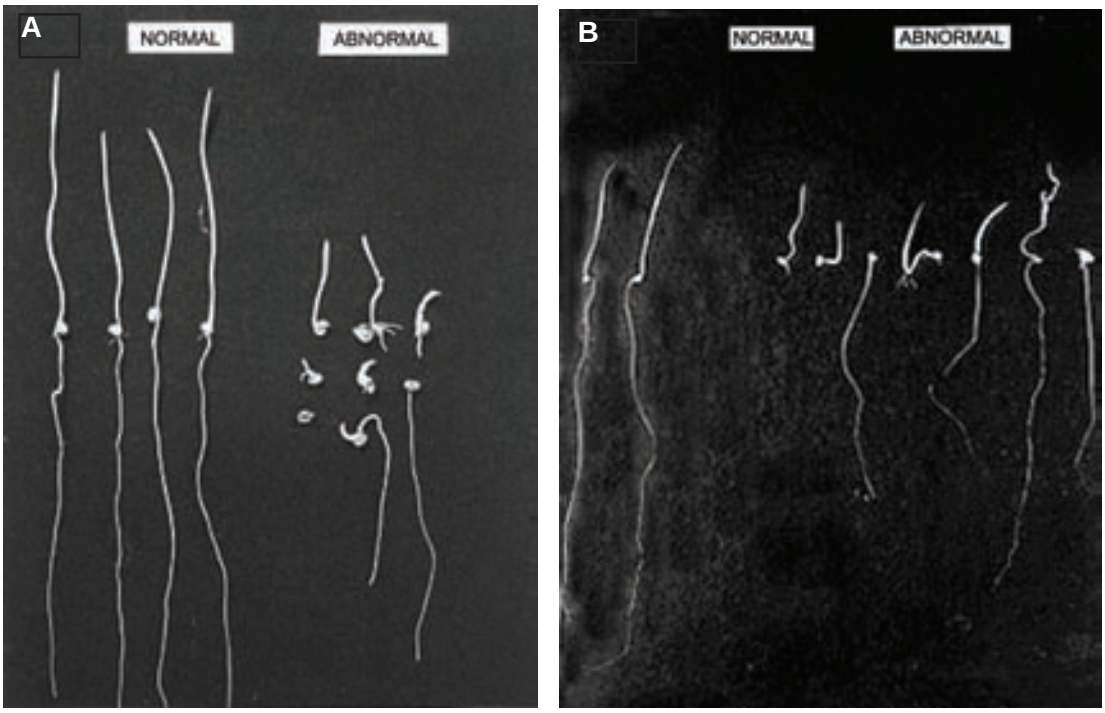

Figure 4D.1.3.3. Normal and abnormal seedlings of sorghum (A) and pearl millet (B).

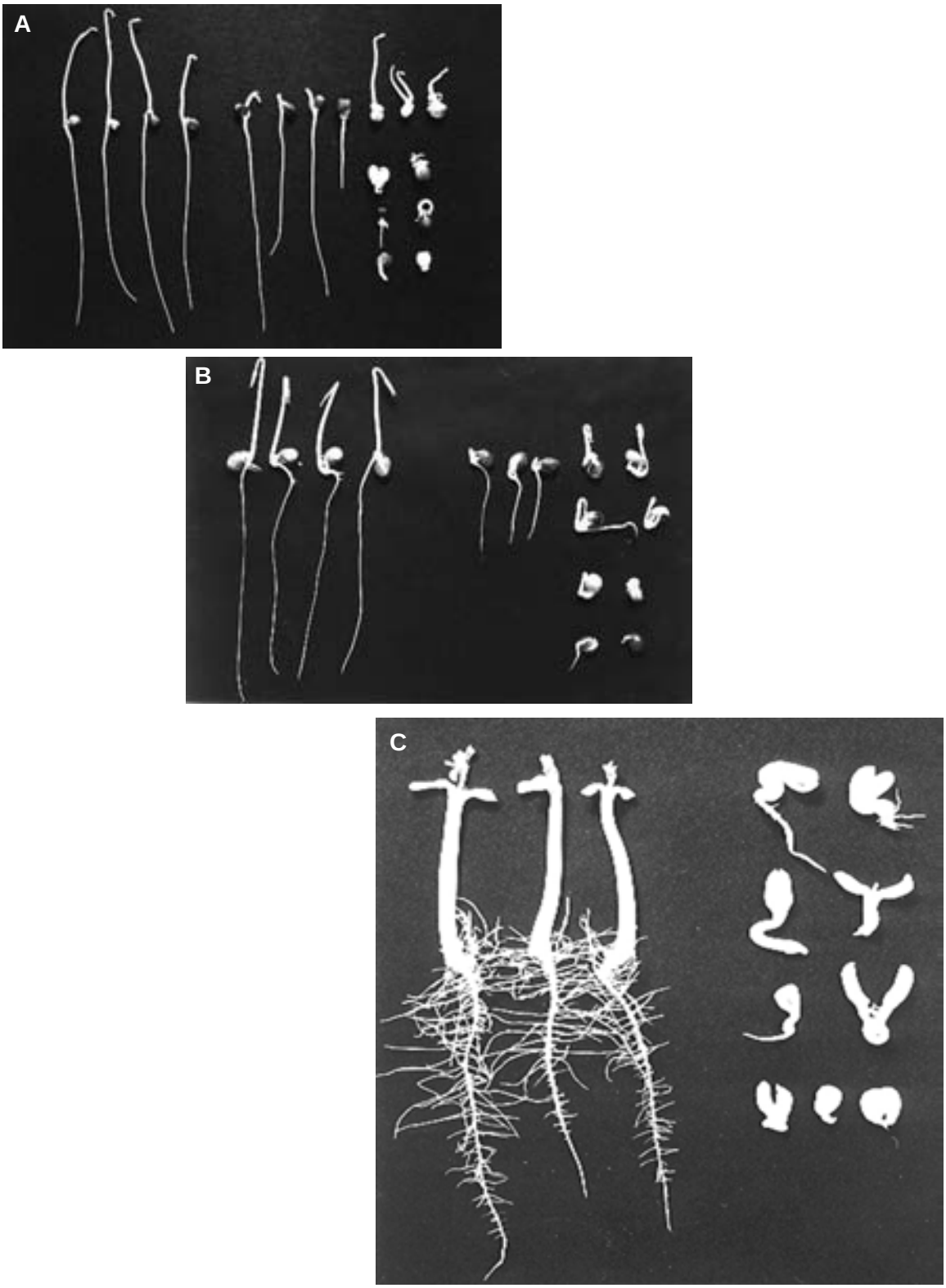

Figure 4D.1.3.4. Normal (left) and abnormal (right) seedlings in chickpea (A), pigeonpea (B) and groundnut (C).

4.D.2. Topographical tetrazolium test for viability

The tetrazolium test can be used as a backup procedure to germination tests in genebanks.

It can be applied to firm seeds, which have failed to germinate at the end of germination test.

The tetrazolium test procedure includes the following steps:

Preconditioning

- Remove the seed covering structures (glumes, etc).
- Precondition the seeds by first soaking in water or by placing them on a moist medium at 30°C.

No preconditioning is necessary when nongerminated seeds are evaluated at the end of a germination test.

Staining

- Bisect the seeds longitudinally through the embryo with a razor blade.
- Discard one-half of the seed and place the other half in the staining solution at recommended concentration (Table 4D.2.1) in a glass vial.
- Place the vials in an incubator maintained in the dark at recommended temperatures and duration (Table 4D.2.1).

Table 4D.2.1. Concentration, temperatures and period of staining with tetrazolium solution.

Crop	Preconditioning	Staining
Arachis hypogaea	Imbibe or soak, 18 h	1%, 40°C, 24 h
Cicer arietinum	Imbibe or soak, 18 h	1%, 30°C, 24 h
Cajanus cajan	Scarify seed, imbibe or soak, 48 h	1%, 30-35^{0}C, 24 h
Pennisetum spp.	Imbibe or soak, 6–8 h	0.5–1%, 30°C, 6–24 h
Sorghum spp.	Imbibe, 16 h, 30°C	0.5–1%, 30°C, 3–24 h

- After staining, wash the seeds several times in distilled water to remove excess stain.
- Immerse the seeds in lactophenol (1 L of lactophenol prepared from 200 ml phenol, 200 ml lactic acid, 400 ml glycerine, and 200 ml water) solution for 1–2 h before evaluation of the seeds.
- Evaluate the seeds for staining pattern under a low power binocular microscope.
- ***Viable tissues stain bright red. Pink and very dark red stains are indicative of dead tissue.***

- Classify the seeds into three categories depending on staining pattern:
  - completely stained and viable seeds,
  - completely unstained seeds that are nonviable, and
  - partially stained seeds.

Preparation of 1% tetrazolium chloride solution

The tetrazolium solution should be between pH 6 and 8 to achieve best results.

Prepare 1 L of buffered 1% tetrazolium chloride* solution as follows:

- Dissolve 3.631 g KH_2PO_4 in 400 ml of distilled water
- Dissolve 7.126 g $Na_2HPO_4.2H_2O$ in 600 ml of distilled water
- Mix the two solutions to prepare the buffer
- Dissolve 10 g of 2,3,5,-triphenyl tetrazolium chloride in the 1 L of buffer solution.

* To dilute the 1% tetrazolium buffered stock solution to produce 0.5% tetrazolium solution, mix one part of the stock solution with one part of distilled water.

The tetrazolium test is not an absolute test of seed viability.

To gain confidence the test must be first calibrated with the results of germination test for each species.

4D.3. Seed vigor tests

Vigor is the sum total of all those properties in seed which upon sowing result in rapid and uniform production of healthy seedlings under a wide range of environments, including both favorable and stress conditions. Vigor tests supplement information about seed quality.

Selected tests for vigor

Speed of germination

Speed of germination is an important measure of vigor. It depends on the time taken to reach 50% germination at constant temperature. Seeds with low vigor take longer time to germinate.

- Place 25–50 seeds over filter paper (Whatman No. 1) moistened with 4 ml distilled water inside a petri dish.
- Count and remove the germinated seeds every 12 h. Germination is considered to have occurred when the radicle protrudes by 2–4 mm.

- Calculate germination index using the equation $\sum(t \times n)/\sum n$, where n is the number of germinated seeds and t is the number of hours from the beginning of the germination test (Annexure 4D.3.1).

Seedling growth test

Measurements of seedling growth (root and shoot) at specific number of days after sowing give an indication of their vigor (Annexure 4D.3.1). Slow seedling growth (shorter roots and shoots) indicates low vigor.

- Conduct the germination test as described earlier and measure the length of the root and shoot.

The seedlings may be cut and dried at 110°C for 17 h to record their dry weights, which is more for better quality seeds.

Membrane integrity

The test is based on measuring the concentration of leachates by electrical conductivity (Annexure 4D.3.1). Low–vigor seeds generally possess poor membrane structure. When such seeds are soaked in water, greater electrolyte loss occurs, leading to higher conductivity of water. The test is mainly used for grain legumes.

- Soak 10 seeds in 50 ml of distilled water in a beaker at room temperature.
- Measure leachate conductivity after 24 h using a digital conductivity bridge.
- Record the reading in $\mu S\ ml^{-1}$ water g^{-1} dry weight of the seed sample.
- A lower reading indicates seeds with higher vigor and vice versa. It is important to note that many species (eg, legumes) have seeds that are impermeable or only slowly permeable to water. This can affect the leaching of electrolytes from seeds in a conductivity test.

4E. Seed health testing

Seed borne fungi such as *Alternaria, Fusarium, Penicillium, Aspergillus* and *Rhizopus* spp. affect longevity during storage. Curators should ensure that seeds prepared for long-term conservation are free from the seed borne pathogens. The methods employed to detect the pathogens are referred to as seed health testing methods.

The commonly used seed health testing methods are:

4E.1. Visual examination

Seeds are examined under an illuminated magnifying lens (2×) or under low–power stereo–binocular microscope (Fig. 4E.1.1). By this method, it is possible to detect sclerotia, smut balls, fungal spores and other fructifications such as pycnidia, perithecia, etc.

4E.2. Blotter test

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

Figure 4E.1.1. Seed health testing in laboratory using binocular microscope.

- Line the lower lid of the petri dishes with three layers of blotter paper moistened with sterile water.
- Drain off excess water and place 20–25 seeds manually with a forceps.
- Evenly space the seeds to avoid contact with each other.
- Incubate the seeds under near ultraviolet light in alternating cycles of 12-h light/darkness for 7 days at 20 ± 2°C.
- Examine the petri dishes under a stereo-binocular microscope for fungi developing on the seeds.

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

4E.3. Agar plate method

This is the most common method used for identification of seed borne fungi.

- Prepare the medium by mixing Potato Dextrose Agar (PDA) powder with appropriate quantity of water.

- Sterilize the mixture in an autoclave at 121°C for 15–20 minutes with 15 lb pressure and cool to about 50°C.
- Carefully pour the mixture into petri dishes by lifting the lid enough only to pour in the agar to avoid contamination.
- Allow it to cool and solidify for 20 min.
- Surface-disinfect the seed by pre-treating for 1 min in a 1% sodium hypochlorite (NaOCl) solution prepared by diluting 20 parts of laundry bleach (5.25% NaOCl) with 85 parts of water.
- Place about 10 seeds (depending on size) on the agar surface with a forceps.
- Incubate the petri dishes at 20–25°C for about 5–8 days.
- 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 to the autoclaved agar medium after it cools to 50–55°C.

4E.4. Seed health standard

- Examine each seed for the presence of pathogens.

If the percentage of seeds infected by one or more of the following fungi is >5%, the seeds are unsuitable for conservation as base collection:

Alternaria, Aspergillus, Cladosporium, Curvularia, Fusarium, Macrophomina, Penicillium Phoma and Rhizopus spp.

4F. Seed packing

Seed packaging involves placing the dry seeds of an accession into a container for storage. Seeds are packaged to:

- Prevent them from absorbing moisture from air
- Avoid mix up of individual accessions
- Prevent contamination by insects and diseases.

Pack the seeds immediately after drying.

4F.1. Types of containers

Different types of containers are available for packaging. The choice depends on storage conditions and species. The packing material should be impermeable to moisture and suitable for long-term use. If the relative humidity of the storage room is not controlled, it is imperative to use moisture proof containers.

Some frequently used containers in genebanks are: glass bottles, aluminum cans, aluminum foil packets and plastic bottles.

- Glass bottles are good but fragile.
- Aluminum cans are difficult to reseal once opened.
- Aluminum foil packets can be resealed and occupy less space. However, seeds with sharp projections can pierce the packets and render them moisture permeable. The packets used for conservation therefore, should have sufficient strength to withstand piercing.
- Plastic bottles are moisture resistant but not moisture proof. So they should be used with caution if relative humidity is not controlled.

The Bioversity International (BI), formerly the International Plant Genetic Resources Institute (IPGRI) has recommended the following specifications for aluminum foil packets to be used for genebanks:

- An outer layer of 17 g m^{-2} Melinex, 4 g m^{-2} lacquer,
- A middle layer 33 g m^{-2} (12 μm) aluminum foil, 4 g m^{-2} lacquer, and
- An inner layer of 63 g m^{-2} polyethylene.

Always obtain containers from a reliable manufacturer. The quality of containers may vary with batches, therefore test each batch for quality before using. When using laminated aluminum foil packets, ensure that the packets are adequately sealed after filling.

Simple test for determining the quality of containers

The quality of the containers and sealing can be tested as follows:

- Fill the container with regenerated silica gel and seal it.
- Accurately determine the weight of the container with an analytical balance.
- Keep the container over water in a desiccator for about a week.
- Remove the container from desiccator and allow the surface to dry.
- Weigh the container and record the change in weight, if any.

  The weight of the container remains constant if the container is moisture proof and the sealing is good. Increase in weight of the container indicates poor quality of the container or inadequate sealing.

- Adjust sealing time and repeat the test to confirm the quality of the container.

Alternatively, the container quality can be tested by filling it with water and holding over silica gel in a desiccator or a ventilated oven at 40°C. Change in weight of the container indicates its poor quality sealing.

At ICRISAT genebank,

- Active collections which are frequently sampled for use are stored in:
  - rust–proof aluminum cans with screw caps and rubber gaskets for sorghum, pearl millet, chickpea, pigeonpea and small millets (Fig. 4F.1.1A), or
  - large size plastic bottles with inner lid and screw cap for groundnut (Fig. 4F.1.1A).

- Base collections conserved for long-term are stored in re-sealable laminated aluminum foil packets* (Fig. 4F.1.1B)

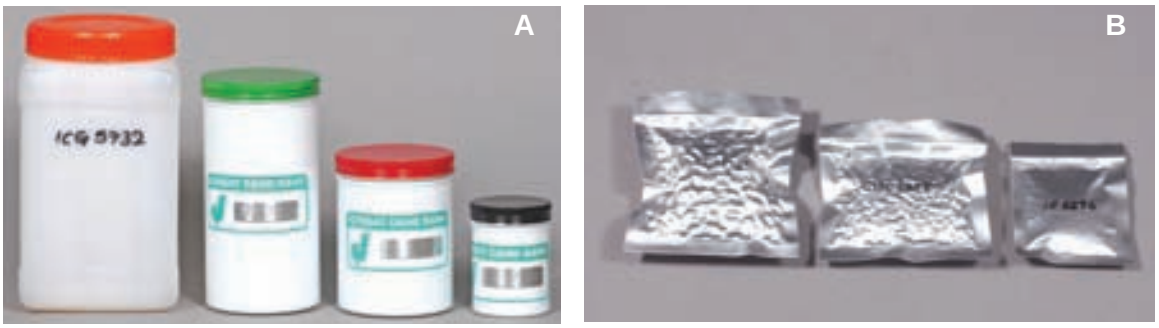

Figure 4F.1.1. Containers used in medium-term (A) and long-term (B) storage at ICRISAT.

4F.2. Packing procedures

Base collections:

Prepare and label the aluminum foil packets with computer generated self-adhesive labels (eg, Z-Label Computer Labels). The label should contain the following information:

- Accession number.
- Identity.
- Season of harvest.

Use aluminum packets of the following size to accommodate the recommended minimum sample size for each crop.

Aluminum packet sizes and approximate maximum quantity of seeds stored in base collections at ICRISAT genebank are as follows:

Crop	Packet size (mm)	Approx. seed quantity (g)
Sorghum and pearl millet	100 × 140	75
Chickpea, pigeonpea and groundnut	190 × 160	200
Small millets	110 × 80	25

- Take out a few samples at a time from the drying room to minimize reabsorption of moisture by the seeds.
- Weigh the amount of seed being prepared for storage.
- Fill the container with seeds leaving at least 2.5 cm headspace for sealing.
- Inside the container, place a non adhesive label with:
  - accession number,
  - identity, and
  - season of harvest - to help in identification of the accession if the label outside is lost.

* Barrier Foils Products Co., Hollands Mill, 61 Shaw Heath, Stockport, SK3, 8BH, UK.

- Seal the container at 0.6 mbar vacuum using Audionvac sealer (Fig. 4F.2.1 A and B).
- Check for any deficiencies in packets and in sealing.
- Print the date of sealing on the packet (if available).
- Move the packets into long-term store.

Active collections:

The following type, size and cap color of containers are used at ICRISAT genebank:

Crop	Type of container	Size/Capacity	Cap color*
Sorghum	Aluminum can	10 × 7.5 cm	Red
Pearl millet	Aluminum can	10 × 7.5 cm	Blue
Chickpea	Aluminum can	10 × 7.5 cm	Yellow
Pigeonpea	Aluminum can	15 × 7.5 cm	Green
Groundnut	Plastic bottle	1.5 kg	Orange
Small millets	Aluminum can	7 × 5 cm	Black

* Color code helps in easy identification when seeds of several crops are to be stored in the same room.

- Paste adhesive metallic labels pre-engraved with accession number or use permanent marking pens for labeling the containers.
- Weigh the amount of seed being prepared for storage.
- Place a label with accession number, identity and season inside the container.
- Fill the container with seed and close the cap tightly.
- Move the containers into storage room.

- ***Do not mix seeds from different seasons.***
- ***Keep them separate in cloth or resealable plastic bags within the same container.***
- ***Do not forget to retain a small sample of the original seed to serve as a reference for future verification of accession identity.***

Figure 4F.2.1. Vacuum sealer (A) and packing seeds (B) for long-term conservation at ICRISAT genebank.

ICRISAT Genebank
Moisture content data

Crop/species:
Grinding: Yes/No
Date of testing:

Temperature: 103°/130°C
Drying time: 16 h/2 h/1 h

Accession Number	Rep No	Weight of dish, g (W1)	Weight of dish + fresh sample (W2)	Weight of dish + dried sample (W3)	(W2 - W3) x 100 (W2-W1)	Mean moisture content (%)
	R I					
	R II					
	R I					
	R II					
	R I					
	R II					
	R I					
	R II					
	R I					
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Annexure 4D.1

ICRISAT Genebank

Crop/species:
Accession number:
Date of storage:
Date of testing:

Substrate: BP/TP
Temperature:
Incubation time: 7d

Germination data

Rep		I	II	III	IV	Total	Remarks
No. of seeds		25/50	25/50	25/50	25/50	100/200	
Date	Days						
Total germinated							
Abnormal							
Hard/Dormant							
Dead							

% Germination

Seed vigor studies

Crop: *Date of testing:*

Accession number: *Temperature: 20/25°C*

Date of storage:

Radicle emergence data

Rep No. of seeds tested		I 50	II 50	III 50	IV 50	Total (n)	t x n	Remarks
Date	h/d (t)							
	12							
	24/1d							
	36							
	48/2d							
	60							
	72/3d							
	84							
	96/4d							
	108							
	120/5d							
	132							
	144/6d							
	156							
	168/7d							
Total								

Mean time (t X n)/ n)

Seedling vigor data

Rep I

Seedling no.	1	2	3	4	5	6	7	8	9	10
Root length, mm										
	11	12	13	14	15	16	17	18	19	20
	21	22	23	24	25	Mean				

Rep II

Seedling no.	1	2	3	4	5	6	7	8	9	10
Root length, mm										
	11	12	13	14	15	16	17	18	19	20
	21	22	23	24	25	Mean				

Mean radlicle length (after 5/7d)

Electrical conductivity

Soaking duration: 24 hrs Temperature: 25°C

Rep	I	II	III	IV	Remarks
Seed #/Water (mL)					
EC (µS)					

Mean (µS)

Monitoring

The viability of seeds stored in the genebank decrease slowly during storage. Removal of seeds for distribution and germination testing results in a decrease of seed quantity over time. Hence, the genebank accessions should be continuously monitored both for viability and seed quantity during storage to avoid excessive deterioration or reduction in seed quantity.

8A. Monitoring seed viability

- Monitor the viability of seeds at regular intervals.
- Monitoring interval depends on the species, seed viability at the beginning of storage or in the previous test, and conditions of storage, as shown below:

	Monitoring interval (years)			
	Active collection (4ºC)		Base collection (–20ºC)	
Germination (%)	Sorghum, millets, chickpea and pigeonpea	Groundnut	Sorghum, millets, chickpea and pigeonpea	Groundnut
<85	5	3	10	5
85-95	8	5	15	8
>95	10	8	20	10

- *Active collections* of sorghum, pearl millet, chickpea and pigeonpea and *base collections* of groundnut with initial viability >95% are monitored every 10 years. Accessions with the initial viability between 85% and 95% are monitored every eight years and those with <85% every five years.
- *Base collections* of non oily crops with >95% viability are monitored every 20 years, those with viability between 85 and 95% every 15 years, and accessions with viability <85% every 10 years.
- *Active collections* of groundnut with >95% viability can be monitored every 8 years, accessions with 85–95% viability every 5 years and those with <85% viability every 3 years.

Seed viability is monitored by conducting germination test on a fixed sample size as described in section 4D.

- Identify and prepare a list of the accessions requiring viability testing on a monthly basis, using genebank documentation system.
- Find the location of the containers in storage from inventory database.
- Remove the containers from storage and leave them overnight at room temperature.
- Open the container and draw a sample of seeds needed for the test and close the containers.

- Update the seed quantity in inventory database, deducting the quantity of seeds drawn.
- Conduct the germination tests as described in section 4D.
- Update the germination data in the inventory database.

8B. Monitoring seed quantity

- Seed quantity is best monitored through a computerized inventory.
- Record the weight of the seeds initially transferred to genebank.
- Record all subsequent seed withdrawals for distribution, regeneration and germination testing.
- Update seed stock immediately adjusting all seed withdrawals.

- ***The genebank documentation system can be designed to issue a warning as seed quantity reaches critical level. The genebank curator can stop further distribution of such accessions until they are regenerated.***
- ***The system can generate reports of accessions at regular intervals when the quantity or viability of seeds falls below minimum so that the genebank curator can program the accessions for regeneration.***