# Testing Seed Viability Using Simple Germination Tests

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**Introduction and Background**

Saving your own seeds is a cost-effective way to access crop seed for future planting and to help maintain the planet's plant biodiversity. Whether you plant your own saved seeds, give them away to friends and neighbors, or distribute them through your organization, knowing the viability of your seeds is important. As a follow-up to the article in the January 2011 ECHO Asia Notes, Issue 8, titled "Building your own seed germination chamber for testing seed viability" (www.echonet.org/repository#1003:d:Build%20Your%20Own%20Seed%20Germination%20Cabinet ), this article will explore the details of several low-cost methods for testing seed viability.

**Seed viability** is a measure of the percentage of seeds that are alive after storage. The greater the viability of your seeds, the fewer seeds will be needed to establish a desired number of plants in the field or nursery.

Seed viability can be tested in many easy ways. A seed germination test is probably the most simple: seeds are given the needed resources (air, water, warmth, and light) to germinate and grow into a seedling. Simply place seeds in the soil or in a pot of soil and see how many grow. However, one disadvantage of using soil, pots, and outdoor resources is environmental fluctuation that can cast doubt on the true viability of the seeds (did the seeds fail to germinate because they were dead, or because they were watered erratically, fell victim to fungal attack, got too hot, etc.?).

A dedicated seed germination cabinet like the one described in the aforementioned article (Figure 1) is a great way to provide constant light and temperatures to germinating seeds, and is another good option. However, even without a cabinet and with very few resources, you can still conduct a reliable seed viability test.

**Figure 1: The seed germination cabinet (See EAN, January 2011 for details on its construction).**

**Procedures**

The various methods for determining seed viability all serve to provide seeds with a substrate that makes water available at the proper amount for the seeds to imbibe (take up into themselves) and to germinate. Choose a method based upon your available resources, and remember that you can always use several different methods to compare results.

All seeds have specific light and temperature requirements, but a general rule of thumb is that most seeds will germinate when the temperature is between 20 and 30ºC, sufficient water is present in the substrate, and some amount of light is given to the seeds (See Table 5.1 in "Manual of Seed Handling in Genebanks" for specific seed requirements and more in-depth coverage of seed viability testing).

Seeds for the test should be randomly selected from your entire lot of seeds. International standards for seed testing suggest that 200 seeds be used in a germination test. If this quantity of seeds is difficult to attain, 100 or even 50 seeds may be used. Divide your total number of available seeds by two, so that you will have two replications for your germination test. If you have an abundance of seeds (lettuce, cabbages, tomatoes, etc.), then four replications of 100 seeds will provide very robust results.

**Control of Pathogens**

Pathogenic contamination by fungi, molds, and bacteria is common in seed germination tests and will often result in poor seed germination and false conclusions. Aseptic technique is an important way to reduce contamination by killing and minimizing the presence of pathogens. Clean and disinfect all work surfaces with a 70 to 95% alcohol solution or a 20% bleach solution (don't forget to wash your hands!). Germination containers and forceps should be soaked in a 20% bleach solution for 10 to 15 minutes, or surface sterilized with alcohol. Prevent seeds from touching each other, regardless of what substrate is used, and promptly remove damaged or decaying seeds, recording the number of contaminated seeds.

The easiest way to prevent contamination is by surface sterilization of seeds to be used in the experiment. The concentration of household bleach is usually between 5 and 6% sodium hypochlorite by volume. Prepare a 1% sodium hypochlorite solution by adding 80mL of distilled water to 20mL of household bleach. Soak seeds for 3 to 5 minutes (3 minutes for small seeds and up to 5 minutes for larger, tough seeds, like beans) in enough bleach solution for adequate coverage (Figure 2). Thoroughly rinse seeds 3 to 5 times in distilled water before introducing them to the substrate.

**Figure 2: Lablab seeds soaking in a 1% bleach solution (use a bigger or different container if you need to).**

**Top of Paper Method**

In the top of paper method, seeds are placed on top of substrate paper in containers with snug-fitting lids (to prevent moisture loss). Glass or petri dishes work well for this method. The ECHO Asia Seed Bank uses rectangular plastic boxes with snug-fitting lids. Use a permanent marker to label the containers with the type of seed being tested and the replication number. Sterilize containers as outlined above, and cut substrate paper (we use a double-folded paper towel) to fit in the container (Figure 3).

**Figure 3: Sizing paper towels for petri dishes: cut a folded paper towel in half with sterile scissors, then trim the long edge of the paper towel to fit perfectly in a petri dish.**

With a sterilized hand or an inverted funnel, snug substrate paper into the container (Figure 4). Add an appropriate amount of distilled water (if distilled water is not available, use boiled and cooled water) to completely moisten the paper without soaking it (Figure 5). We have found that typical containers with a double-folded paper towel require from 2 to 6 ml of water, depending upon the size.

**Figure 4: With sterile hands or tweezers, fit cut paper towel into petri dish**.

**Figure 5: A good workstation flow showing labeled petri dishes, syringe for water application, sterile water, and disinfected seeds; from here petris will go directly into their places in the seed germination cabinet.**

Spread the seeds uniformly on the moistened substrate, ensuring that none of the seeds touch each other (Figure 6). Close the lid, and place the container inside a loosely fitting sandwich bag to help ensure additional moisture retention (Figure 7). You want to prevent loss of moisture, but still allow diffusion of oxygen, which the seeds will need when germinating and respiring.

**Figure 6: Lablab seeds distributed on moistened paper towel with tweezers (they don't need to be in neat rows, but they must not touch each other, in case one is contaminated). I used 25 seeds in each petri for my mock experiment, and the rows helped to ensure that all 25 made it into each dish.**


**Figure 7: Closed petri dish with plastic bag loosely sealing it and loose edges tucked under (to preserve moisture but allow air exchange).**

Place the containers in your seed germination cabinet, or in a warm area where some light is present. Every day, or every other day, count, record, and remove germinated seeds. Germination is defined as the clear and unobstructed emergence of the **radicle**, or seedling root, from the seed coat (Figures 8, 9).

**Figure 8: Radicle emergence of amaranth seed after 1 day; these would be counted as germinated and removed.**

**Figure 9: Radicle emergence of lablab seed after 1 day; these would be counted as germinated and removed.**

Substrates will most likely need to be re-moistened during the course of the test; be sure to give containers with the same seed types an equal amount of water, making note of the amounts. Most seed germination experiments should be run for up to 14 days but some grass species may require up to 28 days to germinate. You may decide to run your experiment shorter or longer depending upon the type of seed, and if all seeds germinate. During the test, carefully remove and discard any seeds that show signs of contamination or decay

**Between Paper Method**

In the between paper method, ordinary paper towels are used as the substrate and also act as the container to hold the seeds. Cut a paper towel to size to adequately hold all the seeds of the replication in neat rows on the towel. Using a pencil or permanent marker, label one edge of the paper towel with the type of seed being tested and the number of the replication. Moisten (but do not soak) the paper towel with distilled water.

Next, place the seeds in rows on the moist paper towel, leaving a 3 cm gap from the top and side edges, and ensuring that the seeds do not touch each other. Any extra space should be at the bottom of the paper towel, which will be immersed in water. Cover the seeds with another moist paper towel and roll the paper towels from the non-labeled edge (Figure 10). Use a paper clip or rubber band to hold the newly formed tube together. The seeds will be inside the tube, held by friction between the two layers of moist paper towel.

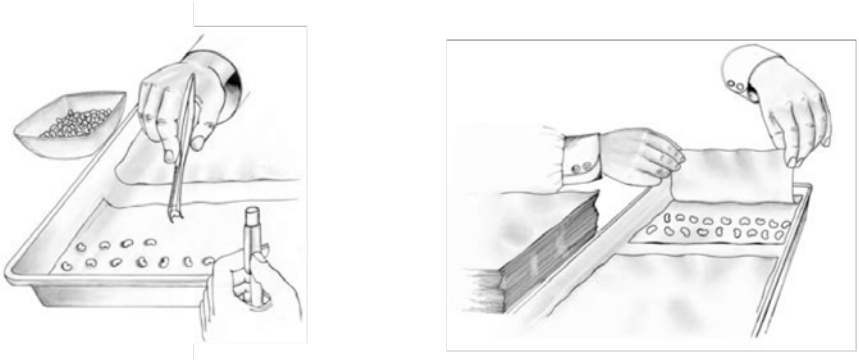

**Figure 10: Placing seeds in the "between paper method."** ***From:*** **Rao et al., 2006.**

Place the bottom of the paper towel tube in a deep-bottomed plastic tray with enough water to ensure that all paper towel tubes are in contact with the water (Figure 11). Place the tray of paper towel tubes in your seed germination cabinet or in a warm area where some light is present. Count, record, and remove germinated seeds by gently unrolling the paper towel tubes and re-rolling after recording.

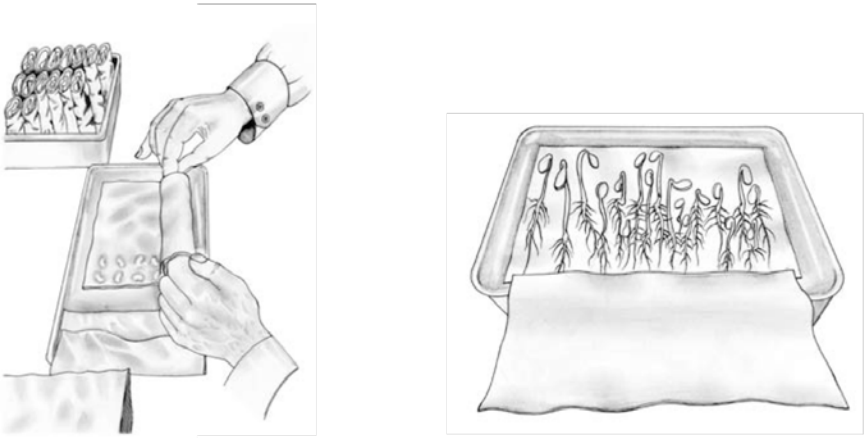

**Figure 11: Rolling and monitoring seeds in the "between paper method."** ***From:*** **Rao et al., 2006.**

Be sure to maintain sufficient water level in the tray; you may also have to spray the tops of the paper towel tubes to keep them moist. Similar to the top of paper method, maintain and continue the experiment for up to 14 days (you may decide to run your experiment shorter or longer depending upon the type of seed, and if all seeds germinate). Take particular care to remove and discard any seeds that show signs of contamination or decay.

**Sand Germination Method**

In the sand germination method, moist sand or other porous media is used as the germinating substrate for the seeds. Start with clean, fine sand that you purchase or steam pasteurize (if possible), and pack into deep-bottomed plastic trays with adequate drainage. Water the sand with distilled water until moist but not soaked (you do not want the sand to evacuate the tray through the drainage holes).

Create equidistant holes in the sand (a pencil or permanent marker works great for this), as deep as the length of the seeds you are testing and spaced three times the length of the seeds. If you are testing more than one type of seed, use a small wooden stake or plastic spoon to mark each row, with the name of the seed being tested and the replication number. Place one seed in each hole, cover them with sand, and water the entire tray. Be sure to water the tray gently so as not to dislocate the seeds (Figure 12). Place the tray in your seed germination cabinet or in a warm area where some light is present.

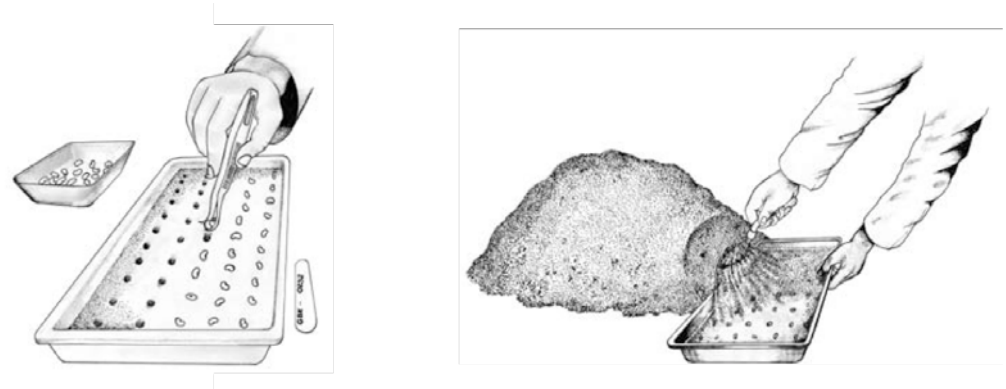

**Figure 12: Planting seeds in the "sand germination method."** ***From:*** **Rao et al., 2006.**

Count and record germinated seeds daily or every other day. However, it is not necessary to remove germinated seeds unless they are crowding each other out. If you choose to remove seedlings, snipping the seedlings at the base will kill the seedling and prevent dislodging non-germinated seeds from the sand.

Keep the sand substrate moist during the course of the experiment, but do not over-water (Figure 13). As per the other two methods, maintain and continue the experiment for up to 14 days, depending upon the type of seed and if all of your seeds germinate.

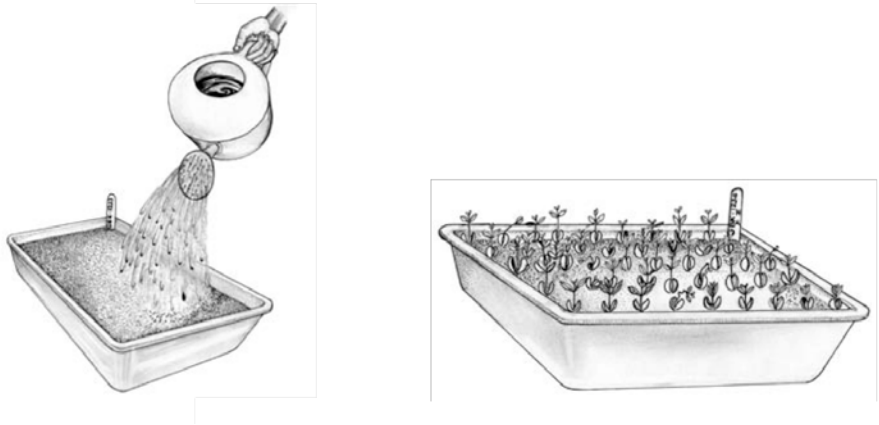

**Figure 13: Watering and monitoring seeds in the "sand germination method." *From:* Rao et al., 2006.**

**Analyzing the Results**

Use the sample data collection sheet (Figure 14) to count and record germinated seeds over the course of your test. Record the total number of seeds you started with for each replicate. Also record the number of germinated seeds and the number of seeds removed from the test due to contamination or decay (these latter we will not count as viable).

**Seed Viability Testing**
**Seed Germination Data Collection and Calculation**

| | | | | Days After Beginning the Test | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Replication | Seed Type | Starting Date | Number of Seeds | 2 | 4 | 6 | 8 | 10 | 12 | 14 | Total Germinated | Germination Rate (%) | Mean Days to 50% Germination |
| 1 | Lablab | 20-Jun | 100 | 33 | 20 | 18 | 10 | 7 | 3 | 0 | 91 | 91 | 4.84 |
| 2 | | | | | | | | | | | | | |
| 3 | | | | | | | | | | | | | |
| 4 | | | | | | | | | | | | | |
| 1 | | | | | | | | | | | | | |
| 2 | | | | | | | | | | | | | |
| 3 | | | | | | | | | | | | | |
| 4 | | | | | | | | | | | | | |

**Figure 14**: **Sample germination data collection sheet with example data in red.**

Percent total germination is a measure of the overall viability of an accession of seeds, and can approximate the number of seeds that will grow into plants when you plant them. At the end of the experiment, add the number of seeds that germinated each day of the trial, divide that sum by the total number of seeds that began the test, and multiply by 100 to calculate the parameter of percentage total germination.

Find the percentage total germination for each replication, so that you can see whether or not the replications differ. If they differ a lot, it would be a good idea to conduct the test again with a greater number of seeds and more replications.

Data that you collect can be used to estimate the length of time a particular seed accession takes to have 50% of its seeds germinate. You can calculate the "mean number of days to 50% germination" as follows: for each day, multiply the number of seeds that germinated by the day you counted them, then add all of those values and divide that number by the total number of germinated seeds. For example, if 12 seeds germinated on day 1, 8 seeds germinated on day 3, and 4 seeds germinated on day 5, for a total germination of 24 seeds, mean number of days to 50% germination would be calculated as: (1x12 + 3x8 + 5x4)/24 = 2.3 days.

**Conclusion**
Testing the viability of your seeds by conducting a seed germination test is an important way to deduce the quality of your seeds, to determine the efficacy of your seed storage methods, and to help you plant the proper amount of seeds. By conducting these simple seed viability tests, you can increase your seed saving efficacy and help to empower farmers in the saving and planting of important genetic diversity.

**Additional Resources**
Gosling, P.G. “Viability testing.” Chapter 24: Seed Conservation. London: Kew Royal Botanical Gardens. *Available:* www.kew.org/science-research-data/kew-in-depth/msbp/publications-data-resources/technical-resources/seed-conservation-science-practice/SCTSIP_chapter24.htm

Rao, N. K., J. Hanson, M. E. Dulloo, K. Ghosh, D. Nowell, and M. Larinde. 2006. Handooks for Genebanks No. 8: Manual of Seed Handling in Genebanks. Rome: Biodiversity International. *Available:*http://www.bioversityinternational.org/index.php?id=19&user_bioversitypublications_pi1[showUid]=3020.

Newman, Y.C., C. G. Chambliss, and M. B. Adjej. 2007. “Rag doll test.” Seed Germination Testing. University of Florida IFAS Extension Publication #SS-AGR-179. *Available:* http://edis.ifas.ufl.edu/ag182.

Douglas, J. L., J. M. Grabowski, and L. E. Daughtry. 2003. “How to use a ragdoll test to estimate field germination.” Plant Solutions for Conservation Needs. Plant Note #5. *Available:* ftp://ftp-fc.sc.egov.usda.gov/GA/PMC/JLW/ragdoll.pdf