

# Plant Propagation Lab Exercise Module 2

## PROPAGATION OF PLANTS FROM SEED SEED VIABILITY TESTING

An introduction to plant propagation laboratory exercises by:  
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In this lab, you will be introduced to two common seed viability tests.

The objectives of this lab are to:

Review the concept of seed viability.

Describe the steps of a TZ seed viability test

Describe the steps of an X-ray viability test

Describe the steps for conducting a rolled paper towel viability test

Provide an opportunity for evaluating the results of seed viability tests using photographs.

Seed viability tests are used to determine the germination potential of a subsample of seeds within a seedlot. Often times when seeds are sold, they are sold with a viability percentage attached to the package and can be interpreted as the maximum possible germination percentage for seeds. Seed viability tests include the TZ or tetrazolium test, X-ray analysis, rolled paper towel test, and the excised embryo test. Each test has its advantages and drawbacks. Here we discuss the TZ test, x-ray analysis, and rolled paper towel test. In TZ tests viable seeds turn red, in an x-ray analysis the fill of seed is visible in the x-ray image, in rolled paper towel tests viable seeds germinate and in the excised embryo test the embryo is removed from the imbibed seed and germinated following standard germination conditions. In the present excised embryo example photo from left to right is a Quinoa grain a Quinoa seed (without pericarp) the Longitudinal midsection of a quinoa seed showing the white perisperm and the last is the Excised embryo where ax is the axis of the hypocotyl and radicle; co are the cotyledons and ram is the root apical meristem. The embryo in image D would be germinated to test for seed viability.

A tetrazolium or "TZ" test is a common laboratory technique used to determine seed viability. During a TZ test, imbibed seeds are placed in a solution and respiring tissues stain red while non-respiring tissues remain partially stained or unstained. A typical TZ test involves immersion of seeds in a 0.1 to 1% solution where TZ powder is dissolved in *pure* water for  $\leq 24$ hr. Post-TZ immersion, seeds are visually assessed for staining color and pattern and are compared to a reference standard to determine which seeds are viable or non-viable. An example of an authoritative reference standard provided by The Association of Official Seed Analysts for 2 genera within Solanaceae shows examples of a positive staining pattern denoted by a plus sign and a negative staining pattern denoted by a minus sign.

Here we have seeds of *Chrysopsis gossypina*, a Goldenaster, which were immersed in a 1% TZ solution for 24 hours at room temperature. Using the top 3 seeds as standards we will calculate the percent of seeds that are viable for each of the 14 seeds with an arrow pointed at them. To do this we divide the number of positively stained seeds by the number of total seeds

and multiply that number by 100.

A total of 8 out of the 14 seeds, or 57% are considered viable. TZ tests can be subjective so it is a good idea to have a full color guide to assist in evaluating positive and negative staining patterns for the species being tested. Additionally, all respiring tissues of a seed will stain red so if seeds are infected with a pathogen a false positive reading could be recorded. For this reason, it is important to maintain clean surfaces and tools and to read the results soon after the staining is completed. Other issues with TZ tests are the need for Personal Protective Equipment and management and disposal of the test materials as hazardous waste. When minimal seeds of a species are available the fact that the test is destructive may also be a factor.

The rolled paper towel test is a relatively simple and low-tech technique that can be used to assess seed viability. In this test seeds are placed within damp paper towels, kept moist in a climate-controlled area, and assessed at a later point in time for germination. Here we describe how to initiate a rolled towel test that can easily be done at home in 6 steps using chickpeas: Step 1 -Place seeds on paper towel, Step 2 -Gently wet until paper towel is damp but not dripping wet, Step 3 -Pinch both ends and carefully roll in a tube shape, Step 4 -Fold as needed to fit in container, and Step 5 -Place in a container to maintain moisture and label with the species, date, and number of seeds.

#### Practice reading viability tests

The following slides will provide you with opportunities to practice reading the various types of seed viability tests.

The final step of the rolled towel viability test is to calculate the viability percentage. To do this we divide the number of normal, germinated seeds by the number of total seeds and multiply the result by 100. Seeds are considered viable when the radical protrudes through the seed coat.  $\text{Viability} = (\# \text{ normal, germinated seeds} / \# \text{ total seeds}) \times 100$

Using the standard provided on the left determine the viability percentage of the seeds pictured. Plus signs indicate positive viability while minus signs indicate seed is not viable. The seeds are of the iconic FL native dune-building grass, *Uniola paniculata* or seaoats.

**Answer=50%.** On the left are two red stained viable seeds and on the right are two unstained non-viable seeds. In an x-ray analysis, standard x-ray equipment is used to produce photograph showing if seeds are filled with nutritive tissues and embryos or whether they are empty or deformed. While not a direct measure of viability since filled seed may be dead, it is still useful in determining the quality of a seedlot. Here filled seeds appear as white while empty seeds appear transparent, only surrounded by a seed coat. 10 of the 15 seeds or 67% of seeds are filled. Additionally, this analyses is not destructive and may be ideal for seeds of limited number or of very high value.

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Place the numbers in order of how they should be performed when conducting a rolled towel test using this example of alfalfa sprouts. **Answer – 4, 2, 3, 1, 5**

Calculate the viability of the rolled towel test using alfalfa sprouts. Hint: a total of 25 seeds are used and seeds are arranged in 5 rows of 5.

A total of 22 out of the 25 seeds germinated for a viability of 88%. There are three seeds that are negative for germination.

Calculate the percent fill for these seeds of the FL native sunflower, *Pityopsis graminifolia* (narrowleaf silkgrass) in this x-ray photograph. Hint: A total of 22 seeds are individually noted by arrows.

Thick blue arrows identify a total of 12 filled seeds while thin orange arrows identify 10 empty seeds. This seed lot has a seed fill of 55%.

### **Lab exercise**

- Read the results for the TZ and rolled paper towel tests in the images provided by your lab instructor.
- Calculate the viability for each seed lot and graph the viability of both viability tests for all four seed lots in the same graph.
- Provide a summary of the results and discuss the experience of reading each type of viability test. Be sure to indicate if you can draw the same conclusions about the seed lots with both test methods.