

6.1 Types of dormancy

Dormancy can be separated into two broad groups: *exogenous dormancy* and *endogenous dormancy*. *Exogenous dormancy*: in which either external environmental components (light, water, gases, temperature) are not available to the seed or physical embryonic expansion and growth is restricted because of impermeable or high density seed coat properties. *Endogenous dormancy*: in which inherent physiological properties of the seed prevent viable seeds from germinating. *Endogenous dormancy* is the more prevalent of the two forms of dormancy. Within these two forms of dormancy are several subtypes:

6.1.1 Exogenous:

- Seed coat impermeability
- Mechanically resistant seed coat

6.1.2 Endogenous:

- Immature embryo (rudimentary dormancy)
- Embryo dormancy (physiological dormancy)
- Inhibitors

6.2 Degrees of dormancy

Seed can have varying degrees or a combination of dormancy types. Therefore, different methods and techniques need to be used to determine dormancy. TZ stains both dormant and non-dormant seed that are viable. These data supply information on the total viability. Paired with germination data, the TZ test yields complete viability information. While the germination test results provide a percentage of seeds that are immediately germinable, the TZ test indicates the percentage of seeds that are viable. The difference represents the percentage of seeds that are dormant. A TZ test can also be performed on ungerminated seed remaining at the conclusion of a germination test to determine the percentage of dormant seed.

6.3 Deep dormancy

With certain seed types, including many wildflower and native range species, the degree of dormancy is so pronounced that staining may require days instead of hours. One method for staining dormant seed is to expose imbibed seeds to drastic temperature alternations (between 5-35° C) as a pretreatment. Another method for staining deeply dormant seed is to precondition the seed with water, cut the seed and place it in a 200-500 ppm gibberellic acid solution (GA₃, a plant growth hormone) at 5° C for approximately 16 hours. Then continue with the prescribed preparation and staining method for that seed type. A stronger concentration of TZ solution may shorten the time for staining dormant seed.

6.4 Hard seed and scarification

Hard seeds require scarification (disruption of the integrity of the seed coat) to allow TZ uptake. Scarification may be performed by mechanical or chemical means. *Mechanical scarification* can be accomplished with sandpaper, piercing needles, or other cutting implements. *Chemical scarification* is accomplished by placing dry seeds in a strong acid or base for an appropriate period of time (see section 8.3.5). Care must be taken to ensure that the scarification process does not damage the seed, decreasing viability.

The percentage of hard seed present in a sample is valuable information; the percentage of hard seed remaining after initial imbibition is determined and reported in addition to the percentage of viable seed, as in a germination test (see 4.2 (d), *AOSA Rules for Testing Seed*). When viability of the hard seeds is

to be determined, the hard seeds are then scarified, imbibed, and tested according to staining and evaluation procedures. If the hard seed percentage is not needed, results are obtained more quickly by scarifying all the seeds prior to preconditioning.

7. EQUIPMENT AND SUPPLIES

Tetrazolium tests can be conducted with a variety of equipment and supplies. The equipment need not be extensive or expensive, and the items selected are often a matter of personal choice and availability. The type of equipment is dependent upon: a) the kinds of seed to be tested; b) the size, scope, and volume of the testing program; and c) the timeliness of the testing procedures. Equipment and supplies may include:

7.1 Staining dishes -

Syracuse watch glasses or 1 dram glass vials for staining grasses, clovers, and other small seeds; petri dishes (both small and large size) for intermediate size seeds; beakers of a 200-250 ml capacity for staining large seeds such as corn and beans. Test tubes work well when testing a small number of seeds.

7.2 Cutting, piercing, cracking devices -

Single-edged razor blades, scalpels and dissecting knives for cutting seed; sharp sewing or dissecting needles and probes for piercing, teasing and manipulating the seed; nail clippers, vises, hammers, drills, files, fine scissors and nutcrackers for cracking or removing pieces of tough or hard seed coats.

7.3 Forceps/tweezers -

For handling and manipulating seeds. Also used in the removal of seed coats.

7.4 Visual aids -

Magnification - A stereoscopic dissecting microscope is recommended for examining small seeds, while a hand lens or magnifying glass will give satisfactory magnification for larger seeds.

Light - A constant source of high-intensity light placed near the magnifying unit enhances clarity and accuracy during seed preparation and evaluation.

7.5 Conditioning (seed moistening) media -

Germination blotters; filter paper; paper toweling, dishes/vials/beakers of water or dilute hydrogen peroxide.

7.6 Temperature control units -

Heat - Ovens, germinators, and growth chambers that can maintain a temperature between 20-40° C for a prolonged period of time. Vacuum ovens that can maintain a temperature maximum of 40° C and a partial vacuum pressure of 150 mm Hg (mercury) can be used to accelerate the staining reaction. (Equipment for doing this is available commercially.)

Refrigeration - A refrigeration unit maintained at a temperature near 5° C is useful in prolonging the seed quality and staining integrity when test evaluation must be delayed. Also, refrigeration of tetrazolium solutions will increase shelf life. A 5° C temperature can be used where a cold imbibition is recommended in preconditioning.

7.7 Miscellaneous items -

Medicine droppers for removing tetrazolium solution after the test is completed. Artist brushes, rectangular glass plates, 85% lactic acid, or glycerol for clearing seed coat pigmentation in many small-seeded grasses. Dilute hydrogen peroxide used to bleach seed coat pigmentations. Lab safety apparatus: fume hoods, exhaust system, eye goggles, latex gloves, first aid kit.

7.8 Check sample to monitor TZ solution-

Seeds such as wheat or barley, from lots known to have high germination, can be used as a check sample to monitor the TZ solution. A paired germination/tetrazolium test can be conducted for comparison.