OREGON STATE UNIVERSITY SEED LABORATORY

TETRAZOLIUM TEST (TZ)
A FAST, RELIABLE TEST TO DETERMINE SEED VIABILITY

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THE NEED FOR FAST VIABILITY TESTS

The Seed Industry needs sound information about the viability of seed lots within a short time to make fast decisions in marketing the seeds. The TZ test, one of the most significant discoveries in seed testing in the 20th century, provides an answer. It determines the percentage of viable seeds within a sample, even if seeds are dormant. This is particularly useful for freshly harvested seeds that possess high levels of dormancy such as some grasses and native species. The results of the TZ test indicate the amount of viable seeds in a sample that are capable of producing normal plants under suitable germination conditions.

While germination test takes 3-4 weeks to be completed in most grass species, a TZ test can be finished within 24-48 hours. It is no surprise that this test is increasingly gaining a wide recognition as a fast method to determine seed viability of field crops, grasses, vegetables, flowers, shrubs, trees, and native seeds throughout the world. With the fast pace of the dynamic grass seed industry, there is no doubt that the TZ test will become a more popular technology in the 21st century.

It is worthy to note that Oregon Seed Certification Program allows tagging seeds based on the TZ test until the germination test results are available. The exceptions are ryegrass and fine fescues because of the fluorescence test and the ammonia test requirements. High correlations between TZ and germination test results were observed in non-dormant seeds.

THE PRINCIPLE OF THE TEST

The TZ is a biochemical test, which differentiates live from dead seeds based on the activity of the respiration enzymes in seeds. Upon seed hydration, the activity of dehydrogenase enzymes increases resulting in the release of hydrogen ions, which reduces the colorless tetrazolium salt solution (2,3,5-*triphenyl tetrazolium chloride*) into a chemical compound called *formazan*. Formazan stains living cells (respiring) with a red color while dead cells (not respiring) remain colorless. The viability of seeds is interpreted according to the staining pattern of seed tissues.

PROCEDURES

The main steps in conducting a TZ test are:

1. Hydration: seeds must be completely imbibed in order to activate respiration enzymes. This process is needed to release hydrogen ions.
2. Cutting or puncturing: This process permits the access of the TZ solution to the internal tissues of seeds. For some grasses, e.g., bentgrass and Kentucky bluegrass, piercing seeds is performed under the microscope for accuracy. For fescues and ryegrass cutting is performed under a magnifying lens. Knowledge of morphology of various seed species is essential for appropriate cutting and piercing of seeds. Preparing grass seeds for TZ test is somewhat time-consuming compared to soybean or corn because of the size of the seeds.
3. Staining: Seeds are placed in a TZ solution (0.1-1.0%) for a period of time as indicated in the AOSA TZ Handbook. During this process hydrogen ions reduce the colorless TZ solution to red formazan, which stains live tissues with red color while dead tissues remain unstained (Figures 1,2, and 3).
4. Evaluation: Critical evaluation of the TZ staining pattern and intensity is needed for accurate interpretation. For reliable evaluation, seed analyst should be familiar with the structure and the anatomy of the seeds to identify the location of the embryos and determine their staining pattern. In some grasses, lactic acid is used to allow for a clear vision of the internal tissues through the seed coat.

The Tetrazolium Testing Handbook of the Association of Official Seed Analysts (AOSA) has detailed techniques for conducting TZ test for a wide range of species.

Like any other seed testing method, the TZ test requires special training and experience. A high level of training is not unique to the TZ test; it is a basic requirement in any test such as purity, germination, ploidy, etc.

WHEN THE TZ TEST IS PARTICULARLY USEFUL?

1. When speed is important and quick decisions about the viability levels of a seed lot has to be made on a short notice, whether the seeds are dormant or non-dormant.
2. It is useful to determine the viability of seed species that are hard to germinate and have deep dormancy. Examples are some tree, shrub and native seeds, also during the first few months after harvest in some crops when the dormancy is in the highest level (example, Kentucky bluegrass). A combination of standard germination test and TZ test of the same sample provides information on the germination percentage as well as the percentage of dormant seeds of that sample (i.e., % TZ - % Germination = % dormant seeds; example: TZ result, 92% - Germination result, 78% = 14% dormant seeds).

3. It can be used as a vigor test by classifying the seeds into high, medium, low and non-viable seeds based on the staining pattern of the seeds.

THE CAPACITY AVAILABLE AT THE OSU SEED LAB

The OSU Seed Laboratory has had the capacity to provide TZ testing for many years. This service is available for a broad range of species, including field crops, grasses, vegetables, flowers, trees, and native seeds. For more information, contact the OSU Seed Lab at (541)737-4464 or 737-4799; Fax: (541)737-2126; or Email: seedlab@oscs.oregonstate.edu

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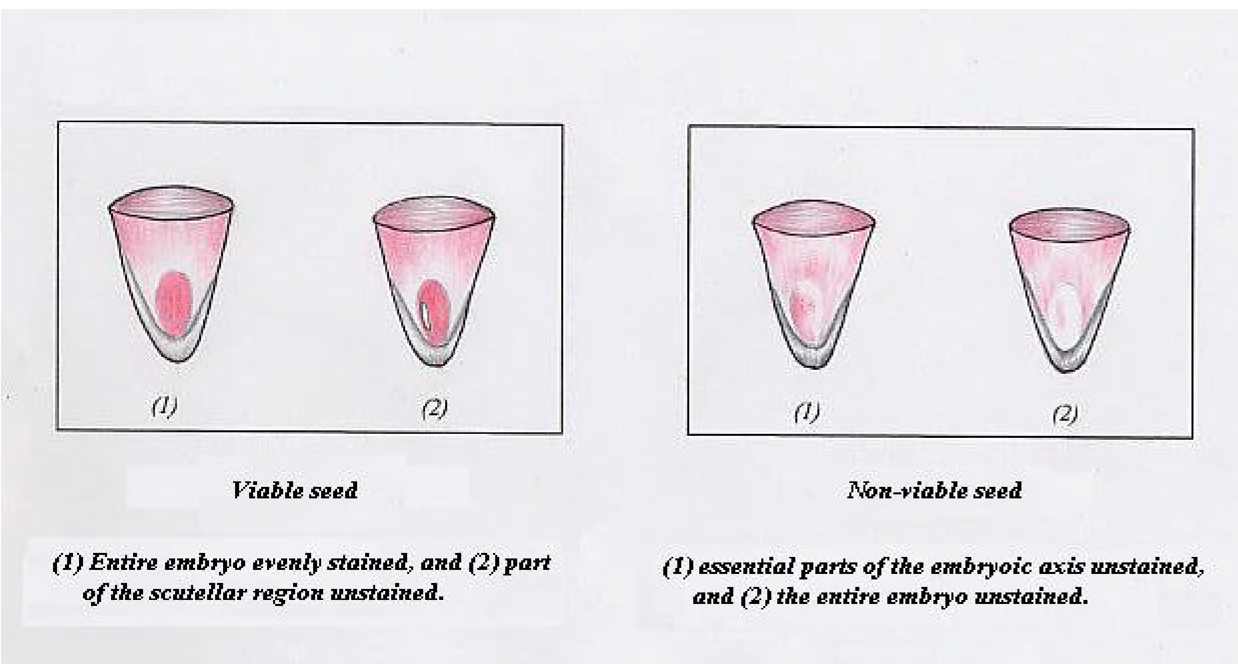

Fig. 1. Examples of TZ staining patterns of viable and non-viable seeds in tall fescue and ryegrass.

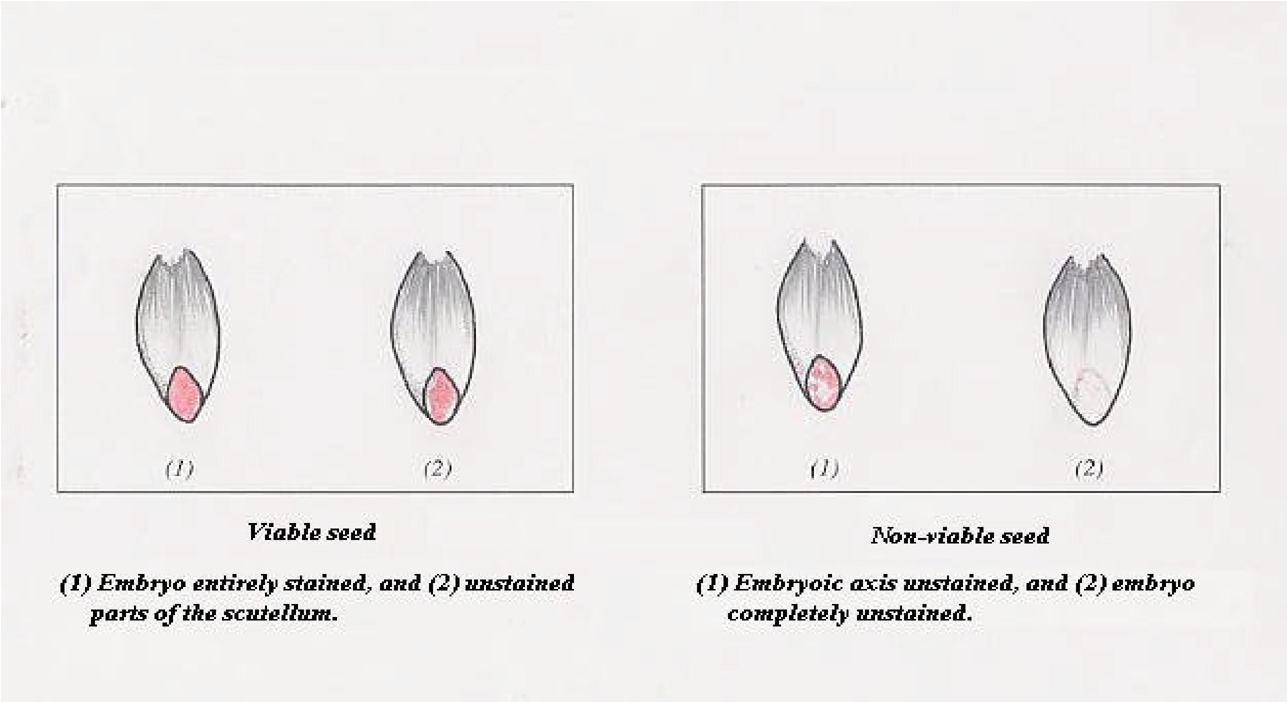

Fig. 2. Examples of TZ staining patterns of viable and non-viable seeds in Kentucky bluegrass and bentgrass.

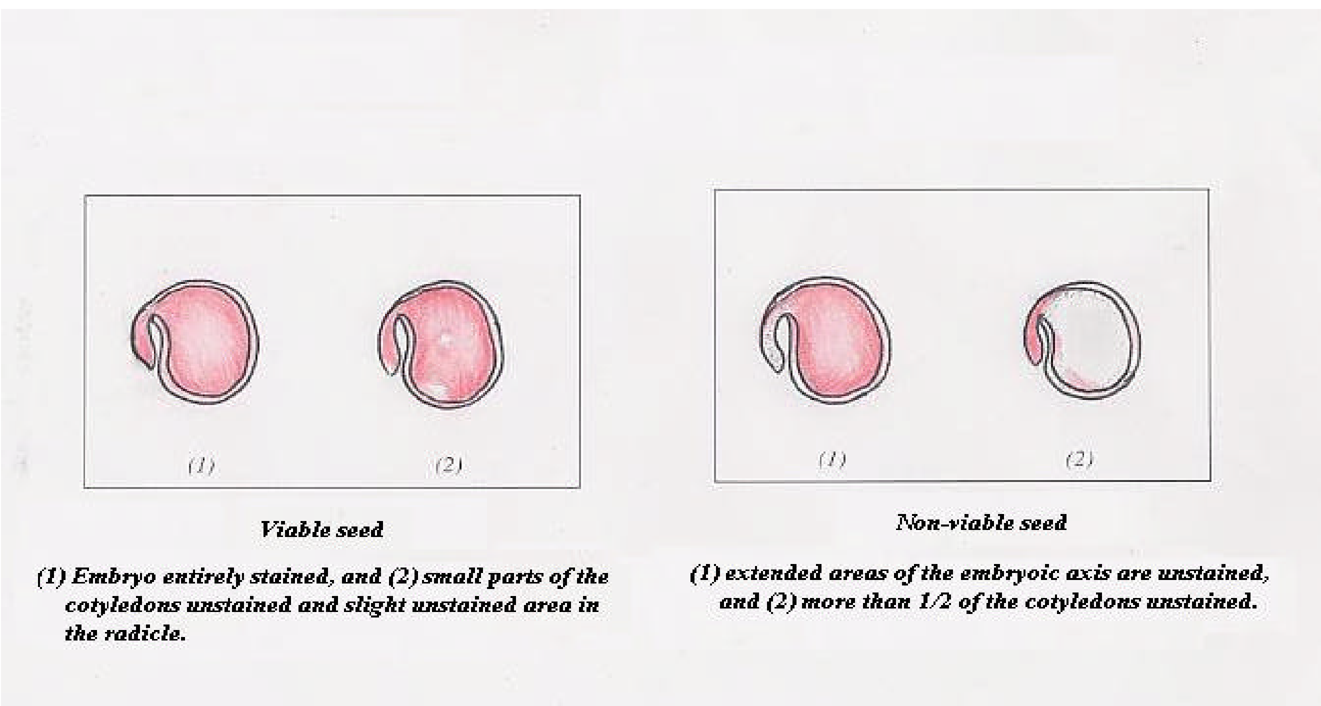

Fig. 3. Examples of TZ staining patterns of viable and non-viable seeds in clover and alfalfa.