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4-1-1967

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Recommended Citation

Moore, R. P., "Seed Facts From Tetrazolium Tests" (1967). *Proceedings of the Short Course for Seedsmen*. 175.

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SEED FACTS FROM TETRAZOLIUM TESTS

R. P. Moore^{1/}

The testing of seeds of various kinds of crops by tetrazolium has provided many unusual and important insights into seed life. Many different kinds of staining symptoms have been evaluated. Many viewpoints have been analyzed. Many new things have been learned about seed life. It is indeed a pleasure for me to share some of these new views concerning seed facts from tetrazolium tests with you.

The tetrazolium method has indeed opened a door to new seed knowledge. It has permitted us to explore the inner parts of various seed structures where so many costly mysteries have remained hidden for such a long period of time. It has permitted us to expose many misconceptions about seed life.

Since time does not permit much discussion concerning man's early efforts to perfect a biochemical seed test, I am making available some reprints of an article entitled, "Tetrazolium Best Method for Evaluating Seed Life," *Seedsmen's Digest* 17(11):38-40, 1966. This reprint will provide many brief glimpses of major events that gradually lead to the development of the first tetrazolium test. After studying the reprint you should realize that tetrazolium testing didn't just happen and that is the main purpose of the reprint. Millions of man hours and dollars were spent by seed physiologists and other scientists as they gradually discovered and refined established segments of truth from which the tetrazolium test eventually emerged.

Many of you are aware that the tetrazolium method has not always been the recipient of the kindest words from its critics. Doubts have often been cast even upon the soundness of judgment of the promoters of the test. In spite of various kinds of criticisms, the method has slowly gained supporters and is now being used for some rather outstanding economic advantages, much to the surprise and regret of the critics. In the meantime the method also has been especially useful in diagnosing causes for seed weaknesses and for recommending corrective measures.

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The Tetrazolium Method

The method involves the use of a colorless solution that reveals, by staining characteristics, the location of normal and weak, as well as of live and dead tissues. Under the guidance of a qualified person, the test is especially informative and useful.

Many persons frequently infer that the test distinguishes dead from living seeds and that it is strictly a staining test. Neither view is exactly correct. The test does distinguish strong, weak and dead tissues within a seed. It reveals in commercial seed lots that the seeds consist of various mixtures of strong, weak and dead tissues. A distinct division between seeds that are completely alive and those that are completely dead is of minor concern. Analysts, however, need to be aware as to which parts of a seed must be alive and intact for a seed to be considered germinative. Analysts must also observe the stained seeds for presence of weak tissues, fractures, bruises, insect damage, etc.

Seed Preparation

If not already moist, seeds must be slowly softened with water prior to testing. Many helpful insights into seed life have been gained even from a careful study of this essential phase of conditioning seeds for a tetrazolium test. We have discovered, for example, that dry seeds of many species tend to fracture readily if moistened rapidly. We have thus gained a good insight as to what has been happening to mature seeds of many crops that were left exposed for even short periods to adverse weather conditions.

Dry seeds of many crops were found to react normally in a tetrazolium or growth test only if first softened slowly in a lightly moistened germination media. It is convenient to do this moistening overnight and at a temperature of approximately 80-90° F.

The seeds thus slowly moistened, react most normally when placed in lukewarm water for completion of swelling prior to staining. After swelling the seeds are suitably prepared for absorption of the tetrazolium stain. Detailed information concerning these and other steps are being made available in reprints of the following articles:

1. Tetrazolium Testing Techniques - Proc. 38th Annual Meeting Soc. Com. Seed Technologists. Pages 45-51. 1960.
2. Tetrazolium Testing Guide - Seed Technologists News 31(2): 18-21. 1962.

An experienced analyst can make many short cuts in techniques and still obtain acceptable results. A new analyst would do well to adhere rather closely to a prescribed set of procedures until the test is mastered. Guidance from a competent analyst can also prove helpful and is strongly recommended.

The Testing Solution

The chemical used is commonly called tetrazolium. The specific chemical powder is purchased under the name 2,3,5-triphenyl tetrazolium chloride (TTC). It costs approximately 25¢ per gram. We purchase our supply of chemical from the Nutritional Biochemicals Corporation, 21010 Miles Avenue, Cleveland 28, Ohio. Other reliable sources are also available.

The testing solution is easily prepared by mixing approximately 1 1/4 grams of powder to approximately 1 pint of tap water. The solution will keep for several months in a dark bottle. Only sufficient solution need be used with each test to cover the seeds. The solution is discarded after each use and the seed covered with water. The cost of the chemical used in one test is of minor importance, oftentimes less than one cent. Labor is the important consideration.

Staining

When water-moistened, living embryo cells or tissues absorb the testing solution, a carmine-red water-insoluble particle gradually develops and intensifies. The hydrogen released during respiration is responsible for the production of this pigment. Dead cells or tissues do not release hydrogen and thus do not stain or at least do not produce a normal color. Living microorganisms will stain, and, if present in sufficient quantities, may at first cause some concern. The color and physical condition of the tissue will then be abnormal.

Living cells or tissues that have been damaged by heat, aging, mechanical injury, etc., will be revealed by color variations that an experienced analyst can soon learn to recognize. The test has many uses. Some of these uses are now to be discussed.

Predicting Potential Germination Percentage

Potential germination percentage refers to seeds that are capable of germinating and producing countable seedling under favorable growth

testing condition. The potential thus establishes the upper limit to be expected in a growth test. Because of dormancy, infection or improperly managed growth tests - a considerable difference may exist between total and potential germination percentages. Such differences do not necessarily mean that the results of either test are wrong. Each test provides a different measurement that must be understood. The failure of a person to understand the true relationship between potential germination percentage and total germination percentage has been the major cause of the majority of criticisms made against tetrazolium tests. This misunderstanding has greatly delayed the use and acceptance of the test.

Evaluating Soundness of Germinative Seeds

The same test used for predicting the potential germination percentage can be used for evaluating the soundness vigor (or energy) of the germinative seed. For a measure of soundness, the individual germinative seeds are separated into two or more classes depending upon the location and nature of weak and dead tissues. Some analysts use only two classes of germinative seeds, namely: 1) sound or essentially sound, and 2) obviously unsound but germinative.

We have found it useful to separate germinative seeds into 5 classes as shown in Table 1.

Table 1. Detailed evaluation of seed soundness revealed by tetrazolium tests.

<u>Germinative seeds</u>	<u>Seed lots</u>		
	<u>A</u>	<u>B</u>	<u>C</u>
	<u>Percentage of Seed in Classes</u>		
1 (Best)	9	0	0
2	58	6	4
3 (Average)	22	24	5
4	3	38	8
5 (Weakest)	2	26	11
<hr/>			
Potential Germination Percentage (1-5)*	94	94	28
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Energy (1-2)	67	6	4
Energy (1-3)	89	30	9

Table 1. Continued.

<u>Non-germinative seeds</u>				
6	(Critical)	4	6	23
7		2	0	39
8	(Dead)	0	0	10

* An estimate of total germination percentage from standard growth tests of fungicide treated seed

This 5-class system requires slightly more effort than the 2-class system. The 5-class system provides the basis for a sliding scale series of evaluations. First, the percentages of germinative seeds are divided into each of the 5 classes. From these percentages various summaries can be made to provide information for 1-2, 2-3 and 1-4 levels of soundness. The 1-5 summary provides potential germination percentage.

Information concerning the percentages of germinative seeds at different levels of soundness can be used in many ways. It furnishes a basis for predicting or establishing:

1. The behavior of different seed lots to different levels of adverse germination conditions.
2. The storage behavior of different lots at different times.
3. The need for effective fungicide treatment.
4. The goodness of seed lots with acceptable germination percentages.

Diagnosing Causes for Seed Weaknesses

No other test approaches the suitability of the tetrazolium test for revealing the causes for seed weaknesses. Aging, internal mechanical injuries, freeze injuries, heat damage, weather exposure damage, insects and disease damage, etc. are commonly recognized. Even calcium deficiency symptoms are frequently noted in peanuts.

Quality Control

By being of short duration and highly informative, the tetrazolium test plays a very important role in quality control programs. The test permits the intelligent acceptance, rejection or handling of seed lots.

It provides excellent guidance in harvesting blending and other processing operations.

The test also stands ready to assist breeding programs in upgrading seed quality. It reveals that slight changes in the nature of seed coats or the shape of seed could be quite helpful in avoiding certain types of critical injuries. Cotton, for example, in the Southeastern area could be greatly benefited by a water impermeable seed coat or even by a stronger seed coat. Recessed embryos would be helpful for peanuts, sorghums, wheat and rye. Snapbeans, soybeans and lima beans quality could be improved by breeding for more water resistant seed coats.

Summary

The tetrazolium test provides a cheap, rapid method for the evaluation of individual structures of individual seeds. The test can be completed within 24 hours, and with moist seed even with 2 hours or less. It requires a minimum of equipment and space.

The tetrazolium test is especially useful and reliable in providing potential germination percentages and germination energy (soundness of germinative seeds). The test can be used to a good advantage in quality control programs and in diagnosing causes for germination difficulties.

Basic to all other factors mentioned, the test can open up many new insights into seed life and can teach even the most experienced seedsmen many new things about the "life" of their business. The tetrazolium test along with growth tests can remove most so-called "mysteries" associated with germination problems. The tetrazolium test can provide a good defense against competitors who have been making regular use of it. The offensive use of the test can provide even greater economic advantages.