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J. C. Delouche

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APPLICATION OF THE TETRAZOLIUM TEST TO COTTON AND SOYBEANS

James C. Delouché

Test methods for rapidly determining or estimating seed viability would contribute much toward greater efficiency in seed processing and marketing operations. Such tests would provide viability information upon which to base sound, timely decisions regarding bulking, blending, processing and marketing of various seed lots.

Rapid viability tests based upon the tetrazolium method are described below for soybeans and cotton. The procedures and methods outlined, if properly applied, will provide reasonably accurate information on germinability within 6 to 8 hours.

Selection of the Seed

The seeds used for the tetrazolium test are selected at random from the pure seed fraction of a purity analysis. If a pure seed fraction is not available, the seeds can be selected at random from a representative sample drawn from the lot of seed. In the latter case, the definition of pure seed used in seed testing should be closely observed, e.g., immature seeds or broken seeds over one-half the size of the original seed should not be discriminated against during selection.

One hundred seeds are generally sufficient for the test provided the seeds are properly selected. These one hundred seeds can be treated as a single replicate or divided into two replicates of 50 seeds each. If ample time and labor is available, two or more replicates of 100 seeds will increase the accuracy of the results.

Soybeans

Preparation of the Seeds

Preconditioning of the seeds before testing is necessary for satisfactory results. The most favorable procedure is to place the seeds between moist towels or blotters overnight (14 - 16 hrs.). However, three or four hours between moist towels are sufficient for preconditioning of the seeds if more time is not available.

The seed coats of most varieties of soybeans need not be removed prior to staining. In certain varieties, however, such as Ototan, Red Tanner and Laredo, tetrazolium will not penetrate the seed coats and it is necessary to remove them prior to staining. In such cases, the seed coats should be carefully removed with fingers and forceps. After removal of the seed coat the seeds are immediately placed in the tetrazolium solution.

1/ Dr. Delouché is Associate Agronomist, Seed Technology Laboratory, Mississippi Agricultural Experiment Station.
Staining of the Seeds

After preconditioning (and removal of the seed coats in the case of some varieties) the seeds are placed in a 1 percent solution of tetrazolium. A small beaker or jar can be used to hold the solution and seeds. The quantity of solution should be sufficient to completely cover the seeds. The tests are then placed at 40°C in darkness. At this temperature the seeds stain adequately for evaluation in 2 - 4 hours depending on the variety. Lower temperatures (or room-temperature) are satisfactory, however, 5 to 7 hours will be required for staining. Regardless of the temperature, the seeds should remain in the solution until a good but not overly intense stain has developed.

Once an adequate stain has developed, the solution is drained off and the seeds placed in clean tap water in a Petri dish or other shallow dish. The seed coats are then carefully removed to permit examination of the staining patterns.

Interpretation (Plate 1)

At the end of the staining period, the seed coats of a majority of the seeds are still intact. These seeds are generally viable; however, the seed coats must be removed and the seeds examined before a definite decision can be reached. Badly deteriorated seeds develop severe ruptures in the seed coats and the cotyledons are cracked or split. The seed coats of other seeds develop slight splits, but no particular significance can be attached to this reaction.

Twenty typical staining patterns are illustrated in Plate 1. The drawings are paired illustrations of both sides of the seed. The symbol N indicates seeds which are capable of producing normal seedlings. The symbol AD indicates seeds which yield abnormal seedlings or are not capable of germination. An explanation of the significance of the various staining patterns depicted is indicated below.

Illustration
No. 1  NORMAL. Seed completely stained; stain usually not perfectly uniform in color.
Nos. 2 - 7  NORMAL. Illustrations depict progressively larger dead areas on cotyledons; capable of normal germination; vigor reduced.
No. 8  ABNORMAL OR DEAD. Seed well stained except radicle; radicle white; root development abnormal.
Nos. 9 - 13  ABNORMAL OR DEAD. Unstained, dead areas at various position on radicle or in the critical region at juncture of radicle, cotyledons and epicotyl.
PLATE I - SOYBEANS

1 N
2 N
3 N
4 N
5 N
6 N
7 N
8 AD
9 AD
10 AD
11 AD
12 AD
13 AD
14 AD
15 AD
16 AD
17 AD
18 AD
19 AD
20 AD
<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>ABNORMAL OR DEAD. Major portion of cotyledons unstained and dead.</td>
</tr>
<tr>
<td>15</td>
<td>ABNORMAL OR DEAD. Seed usually intensely stained; radicle area milky or pearlish in appearance (appears as a cloudy film over stained area underneath).</td>
</tr>
<tr>
<td>16-18</td>
<td>ABNORMAL OR DEAD. Various combinations of milky appearing areas and unstained areas in critical locations.</td>
</tr>
<tr>
<td>19</td>
<td>ABNORMAL OR DEAD. Seed stained abnormally dark red; interfaces of cotyledons usually well stained; cutting through cotyledons reveals deep penetration of stain.</td>
</tr>
<tr>
<td>20</td>
<td>ABNORMAL OR DEAD. Seed completely unstained; white or greenish in appearance.</td>
</tr>
</tbody>
</table>

There are several other points which should be considered in interpreting tetrazolium test results on soybeans. The interfaces of the cotyledons generally do not stain or they stain very faintly. This probably results from a lack of contact between this tissue and the chemical.

In some instances very faint staining of the seeds might be misinterpreted as a complete absence of staining. When soybeans are tested without removing the seed coats, the coats of vigorous seeds apparently restrict the penetration of the chemical so that some areas of the seed stain only faintly by the end of the period. Such areas usually have a faint yellow or pink cast and the tissue is quite firm. However, if there is any doubt the seeds should be placed in a tetrazolium solution for an additional 15 to 20 minutes and re-examined.

Seeds with wrinkled seed coats develop elaborate staining patterns. A well defined network of red and yellow stripes and bands develop and apparently correspond to the wrinkles in the seed coat. The yellow areas should not be misinterpreted as dead areas.

As previously indicated, the seeds do not always stain uniformly. Dark, light and medium red areas occur on the same seed. This reaction is apparently normal. For example, the areas underneath splits developed in the seed coat during absorption are usually more intensely stained than other areas of the seed.

When the methods described above are properly applied, the tetrazolium test will usually yield quite accurate results. It should be pointed out, however, that in exceptional cases, the tetrazolium test can lead to very inaccurate conclusions.

The beginner in tetrazolium testing will find it profitable to make comparative germination and tetrazolium tests until he becomes familiar with the colors and patterns developed in the tetrazolium test.
Cotton

Preparation of the Seeds

Tetrazolium will not penetrate the seed coat of cottonseed. It is, therefore, necessary to remove it. Removal of the seed coat is most easily accomplished if the seeds are soaked in warm water (85 - 95°F) for a minimum of 5 - 6 hours. After the seed coat has been removed, a thin membrane remains around the cotyledons. It is also necessary to remove this membrane for satisfactory staining. Generally, the membrane can be rubbed off with the fingers immediately after removal of the seed coat. A preferable procedure, however, is to soak the decoated seeds for 15 - 20 minutes in water to further soften the membrane before its removal. Care should be exercised during removal of the seed coat and membrane so that the seed tissues are not injured.

While removing the seed coats it will be observed that some seeds are obviously decayed and dead. These need not be tested. However, the number of dead seeds should be noted so that they can be included in the calculations after the test is completed.

Staining of the Seeds

The prepared seeds are placed immediately in a 1 percent tetrazolium solution. They should not be allowed to dry out. A beaker or small jar can be used to hold the seeds and solution. A sufficient quantity of tetrazolium should be used to completely cover the seeds. The tests are then placed at 40°C in darkness. At this temperature the seeds usually stain adequately for interpretation in 2 hours or less. Lower temperatures are satisfactory but the time necessary for staining is greatly increased. Regardless of the temperature, the seeds should remain in the solution until a good but not overly intense stain has developed.

After the seeds have stained satisfactorily, the solution is drained off and the seeds are placed in clean tap water in a Petri dish or other shallow dish.

Interpretation (Plate 11)

Interpretation of tetrazolium tests on cottonseed is difficult. With a little experience, however, it is possible to estimate viability with reasonable accuracy. This situation is not too surprising as the standard germination test is also subject to considerable variability.

Twenty typical staining patterns are illustrated in Plate 11. The drawings are paired illustrations of patterns developed on both sides of the seed. The symbol N indicates seeds which are capable of producing normal seedlings. The symbol AD indicates seeds which yield abnormal seedlings or which fail to germinate. An explanation of the significance of the various staining patterns depicted is indicated below:

Illustration

No. 1 NORMAL. Seed completely stained; stain not overly intense.
Nos. 2 - 8  **NORMAL.** Minor white, dead spots on cotyledons; capable of normal germination; vigor reduced.

No. 9  **NORMAL.** Extreme tip of radicle white; root growth normal or secondary roots develop.

No. 10  **NORMAL.** Extreme tip of radicle white; minor dead areas on cotyledons; capable of germination.

No. 11  **ABNORMAL OR DEAD.** Extreme tip of radicle white; large dead area on cotyledons; abnormal development.

No. 12  **ABNORMAL OR DEAD.** Most of protruding portion of radicle white and dead; abnormal root development.

No. 13  **ABNORMAL OR DEAD.** Major portion of radicle white and dead; small dead areas on cotyledons.

No. 14  **ABNORMAL OR DEAD.** Major portion of cotyledons white and dead; dead area extends into critical region at juncture of hypocotyl and cotyledons.

Nos. 15 - 17  **ABNORMAL OR DEAD.** Major portion of seed white and dead.

No. 18  **ABNORMAL OR DEAD.** Off-type stain; milky appearance (grayish red); cotyledons usually tightly appressed; not expanded.

No. 19  **ABNORMAL OR DEAD.** Seed stained abnormally dark red (purplish red); cotyledons not expanded.

No. 20  **ABNORMAL OR DEAD.** Seed completely unstained; white or greenish yellow in appearance.

Cottonseed usually stains fairly uniformly. Minor injuries resulting from removal of the seed coat sometimes stain dark red.

Normally, the cotyledons expand slightly during the soaking and staining periods. Seeds illustrated in drawings 19 and 20 usually do not expand; the cotyledons remain tightly wrapped and the seeds appear smaller than others.

Because of the difficulties in interpretation of tetrazolium test results, the beginner should run comparative germination tests along with tetrazolium tests. Upon the basis of the results of the comparative tests, the analyst can adjust his procedures to increase the accuracy of his estimate.