

Kind of Seed	Preparation	Concen- tra-tion of Solution (%)	Staining Time at 35°C. (Hrs.)	Evalu- ation Group	Additional Remarks
Smilo	Bisect longitudinally	0.1	2-3	B	
Sorghum allum	Bisect longitudinally	0.1	2-3	B	
Sorghum	Bisect longitudinally	0.1	½-1	A	
Sorghass	Bisect longitudinally	0.1	½-1	B	
Soybean	No preparation	1.0	3-4	D	Hard seeds
Sudan grass	Bisect longitudinally	0.1	2-3	B	
Sunflower	Remove seedcoat	1.0	3-4	E	
Sweet vernal grass	Bisect longitudinally	0.1	2-3	B	
Switch grass	Bisect longitudinally	0.1	2-3	B	
Timothy	Bisect laterally or pierce	1.0	4-6	C	Clear with lactophenol
Tobacco	Remove seedcoat	1.0	4-6	E	
Trefoil	No preparation	1.0	6-7	D	Hard seeds
Vasey grass	Bisect longitudinally	0.1	2-3	B	
Veldgrass	Bisect longitudinally	0.1	2-3	B	
Velvetgrass	Bisect longitudinally	0.1	2-3	B	
Vetch	No preparation	1.0	6-7	D	Hard seeds
Wheat	Bisect longitudinally	0.1	½-1	A	
Wheatgrass	Bisect longitudinally	0.1	2-3	B	
Wildrye	Bisect longitudinally	0.1	2-3	B	

- h. Embryo with yellow or a faint pink cast, if firm in texture, and giving evidence of delayed absorption of tetrazolium.
- i. Less than $\frac{1}{2}$ of cotyledon tissues unstained or non-functional.

A non-germinable seed is one in which one or more of the following types of deterioration are present:

1. The complete embryo or a major portion of it is not stained and is of dull appearance and flaccid, or is of distinctly abnormal color or texture.
2. Broken radicle which is easily separated from cotyledons when slight pressure is applied.
3. Embryo with deep-seated deterioration of cotyledon tissues that extends to inner flat surfaces.
4. Embryo with both cotyledons functionally severed from embryonic axis by fractures or deteriorated tissues, or by transverse fractures or deteriorated areas that cause more than $\frac{1}{2}$ of the total cotyledon tissue to be non-functional.
5. Embryo with extensive surface necroses involving vascular tissues, or extensive mottling of brownish or bluish red and white staining patterns.
6. Embryo with deteriorated areas on hypocotyls that involve more than $\frac{1}{4}$ of the diameter of the stele.
7. Embryo with deterioration of radicle tips that extends upward and beyond the tapering or angular cell-division area of the stele.
8. Embryo with epicotyl or both plumules made non-functional by fractures.
9. Embryo with necroses of plumules, especially frequent in snapbeans, that cause more than $\frac{1}{2}$ of the plumule surfaces to be non-functional. In borderline cases the pair of plumules should be broken loose for observation on both sides.

GROUP E: Dicotyledonous seeds other than legumes (Fig. 10 and Plate VI)

This is a heterogeneous group of species. Although unrelated, all have basically similar seed structures with a radicle, plumule, and two cotyledons. Most have a thick, tough seedcoat or pericarp which must be cut or removed before staining. As in other species, interpretation of staining patterns should be based on the likelihood of essential structures developing normally during germination. In several of these species, tetrazolium test results may be higher than corresponding germination test results if the seeds are attacked by fungi or are dormant.

A germinable seed is one in which:

1. The embryo structures are well developed, intact and of normal red color.
2. Embryo contains no more than the maximum listed for one or more of the following:
 - a. Small necroses on cotyledons in areas other than at juncture of embryonic axis and cotyledons.
 - b. Small necroses at the extreme tip of the radicle.

A non-germinable seed is one in which one or more of the following types of deterioration are present:

1. Embryo completely or mostly unstained.
2. More than extreme tip of radicle unstained.
3. More than $\frac{1}{2}$ of cotyledon tissue unstained or made non-functional by a fracture.
4. Deep-seated necrosis at cotyledon and embryonic axis juncture or on radicle.
5. Purplish-red or greyish-red stain.
6. Fractured radicle.
7. Poorly developed, immature seed.

Figure 10. Criteria for interpreting tetrazolium test results on cottonseed. Illustrations are paired and depict both sides of seed. Black areas indicate stained, living tissue; white areas represent unstained and dead tissue.

- | | |
|-----------|---|
| No. 1 | GERMINABLE. Seed completely stained; stain not overly intense. |
| Nos. 2-5 | GERMINABLE. Minor unstained areas on cotyledons. |
| No. 6 | GERMINABLE. Less than one-third of cotyledons unstained. |
| No. 7 | GERMINABLE. Extreme tip of radicle unstained; minor unstained spots on cotyledons. |
| No. 8 | NON-GERMINABLE. More than one-third of cotyledons unstained; extreme tip of radicle unstained. |
| Nos. 9-10 | NON-GERMINABLE. More than extreme tip of radicle unstained. |
| No. 11 | NON-GERMINABLE. Unstained area on cotyledons extends into region where radicle and cotyledons are attached. |
| No. 12 | NON-GERMINABLE. More than one-third of cotyledonary tissue unstained. |
| No. 13 | NON-GERMINABLE. Seed stained grayish-red, cloudy or milky red color; cotyledons not expanded; seed relatively smaller than germinable seed. |
| No. 14 | NON-GERMINABLE. Seed stained abnormally dark; purplish-red; cotyledons not expanded; seed relatively smaller than germinable seed. |
| No. 15 | NON-GERMINABLE. Seed completely unstained. |

TETRAZOLIUM TESTING HANDBOOK

Tetrazolium testing has been developed to furnish quick estimates of seed viability. Such estimates are useful in facilitating the buying and handling of seeds, testing dormant seed lots, preliminary testing in seed control work, rating seed lots for vigor, supplementing germination tests, and diagnosing causes of seed deterioration.

This handbook has been prepared in response to a need for a uniform set of instructions for tetrazolium testing of seeds. It is written with the assumption that the analyst is already familiar with the art and science of seed testing as exemplified in the Rules for Testing Seeds of the Association of Official Seed Analysts, the International Rules for Seed Testing, and Handbook No. 30, Testing Agricultural and Vegetable Seeds, published by the United States Department of Agriculture. For further information on tetrazolium testing, the reader is referred to the extensive list of references included at the end of this Handbook.

1. SOURCE OF SEEDS FOR TESTING

Careful sampling procedures must be followed in obtaining seeds for testing if the results are to be representative of the viability of the entire lot. Since variability always exists in seed lots, composite samples should be obtained by mixing together samples taken from evenly distributed parts of the lot to be sampled. For free-flowing seed in bags or bulk, a probe or trier should be used. For non-free-flowing seed, representative portions may be taken with the hand.

Detailed instructions on sampling for purity and germination tests which are given in Section 1 of the Rules for Testing Seeds are equally applicable for tetrazolium testing.

2. NUMBER OF SEEDS TO TEST

At least 200 seeds should be tested, in replicates of 100 seeds or less. The seeds should be randomly selected and counted out in replicates before conditioning. Tetrazolium tests require less replication than germination tests because of less dependence on moisture, temperature, light, fungi, and other environmental factors that may cause variation in results. In the case of seeds that are extremely difficult to prepare for staining, or for less precise information, it may be practical to test less than 200 seeds.

3. EQUIPMENT

Tetrazolium tests may be conducted with a variety of equipment without affecting the results. The items selected are often a matter of personal choice and availability. Examples of suitable equipment include:

- (a) Staining dishes: Syracuse watch glasses for staining grasses, clovers, and other small seeds; petri dishes and beakers of 200-250 ml. capacity for staining seeds the size of corn and beans.

- (b) Cutting and piercing devices: Single-edge razor blades are satisfactory for bisecting seeds, since they are inexpensive, sharp, and disposable. Sharp dissecting knives are sometimes used. Sharp sewing needles or dissecting needles are satisfactory for piercing.
- (c) Forceps: For handling seeds.
- (d) Magnifying devices: A stereoscopic microscope is recommended for examining small seeds, while a suitable magnifying lens will give satisfactory enlargement for larger seeds.
- (e) Medicine dropper: For removing tetrazolium solution after test is completed.
- (f) Dispensing bottle: For application of lacto-phenol.
- (g) Needles: Spear-pointed needles or teasing needles for manipulating seeds during interpretation.
- (h) Conditioning media: Germination blotters, filter paper, and paper toweling.

The staining reaction may be accelerated by conducting the test under conditions of high temperature (approximately 45°C.) under vacuum. Equipment for doing this is available commercially.

4. SOLUTIONS

4.1 Tetrazolium solutions:

The recommended tetrazolium derivative is 2, 3, 5-triphenyl tetrazolium chloride (TTC), and is available from several chemical supply houses. Two such supply houses are: Nutritional Biochemicals Corporation, 26201 Miles Road, Cleveland, Ohio 44128, and Fisher Scientific Company, 1458 North Lamon Avenue, Chicago, Illinois 60651.

Several concentrations of tetrazolium solution may be used with comparable results. Conveniently, stock solutions of 1.0% and 0.1% may be maintained. The 1.0% solution is used for seeds that are not bisected through the embryo, while the 0.1% solution is used for seeds in which the embryo is bisected. Other low concentrations such as 0.2% and 0.5% are sometimes used instead of the 0.1% solution.

To prepare a 1.0% solution, dissolve 1 gram of tetrazolium powder in 100 ml. distilled or tap water. To prepare a 0.5% solution, mix 1 part of 1.0% solution with 1 part water, or dissolve 1 gram tetrazolium powder in 200 ml. water. To prepare a 0.1% solution, mix 1 part of 1.0% solution with 9 parts water, or dissolve 1 gram tetrazolium powder in 1000 ml. water.

The pH of the solution should be between 6 and 8 for best staining to occur. Infrequently, a batch of tetrazolium will be obtained which will produce a solution pH of 4 or lower. Solutions of such high acidity will not stain seeds properly.

If the pH of the water is not in the neutral range, the tetrazolium salt should be dissolved in a phosphate buffer solution. The buffer solution is prepared as follows:

Solution 1 — Dissolve 9.078 g. of KH_2PO_4 in 1000 ml. of water.

Solution 2 — Dissolve 11.876 g. of $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ in 100 ml. of water.

Take 400 ml. of solution 1 and 600 ml. of solution 2 and mix them together. In the liter of buffer solution prepared as above, dissolve 10 g of tetrazolium salt. This gives a 1.0% tetrazolium solution of pH 7.0.

Store the solutions in the dark or in an amber colored bottle to prevent deterioration from light. The solutions may be kept for several months at room temperature. The solution used in a test is usually discarded after each test.

4.2 Lactophenol

Lactophenol solution, consisting of 20 parts lactic acid, 20 parts phenol, 40 parts glycerin, and 20 parts water, is used for clearing grasses such as bluegrass and timothy after staining so that the embryo is visible through the lemma and palea. The solution may be conveniently dispensed with a medicine dropper or from a small plastic dispensing bottle.

Warning: Lactophenol is toxic, both on contact and through inhalation. Avoid skin contact and provide ventilation to reduce concentration in the air.

5. TEMPERATURE

Temperatures between 20° and 45° C. (68° and 113° F.) have no effect on accuracy of tetrazolium tests, but staining proceeds faster at the higher temperatures. Tests may be performed satisfactorily at room temperature. If shorter test periods are desired, heat can be supplied by placing the tests in a 30° or 35° C. (86° or 95° F.) germinator or other heating device. Do not use temperatures warmer than 45° C. As a general Rule of Thumb, staining will take place twice as fast at 30° as at 20° C., and twice as fast at 40° (104° F.) as at 30° C.

6. LIGHT

Tests may be placed in subdued light or in the dark for staining; however, light has little effect on the tetrazolium test and may be disregarded as a factor affecting accuracy of results.

7. PREPARATION OF SEEDS FOR TESTING

7.1 Conditioning

Interpretation of staining patterns is facilitated when seeds are softened in water before sectioning or piercing. During this conditioning period, germination processes are initiated and the tissues become less fragile.

Seeds are prepared for sectioning by placing them on top of or between moist blotters or paper towels overnight or by placing them in a beaker of water for 3-4 hours at a warm temperature (30° C.). Seeds are sufficiently conditioned when they are fully imbibed with water and soft

enough to allow a clean slice through the embryo. The overnight method generally is more compatible with seed laboratory routine. Soaking in water is the quicker method and may be desirable when testing in the field, but dry brittle seeds, especially beans, may fracture if placed directly in water. The choice of conditioning method will depend on the speed and accuracy required and the characteristics of the seed.

Seeds of small-seeded legumes and some other kinds do not require conditioning, but may be placed directly in the tetrazolium solution.

When time is of the essence, seeds of grasses and cereals may be bisected and stained without conditioning. Interpretation is often more difficult, however, because of poor sectioning and breaking and crumbling of the embryo, but a satisfactory estimate of viability may still be possible.

Seeds that are heavily treated with red-colored fungicide should be washed before conditioning to prevent the red dye from obscuring the tetrazolium staining patterns.

7.2 Preparation for staining

The seeds must be prepared in such a way that the tetrazolium solution can come in contact with the embryo. The pericarp of grasses is not permeable to tetrazolium, so the pericarp must be broken by sectioning or piercing. Tetrazolium penetrates seedcoats of small-seeded legumes so no preparation is needed. Some other kinds of seeds have thick, tough seedcoats which must be removed before staining.

Seeds generally are conditioned and prepared for staining according to one of the following methods:

METHOD 1: Bisect longitudinally (Figure 1)

(corn, sorghum, small grains, large-seeded grasses)

Condition the seeds overnight between folded moist blotters or by

PREPARATION OF SEED

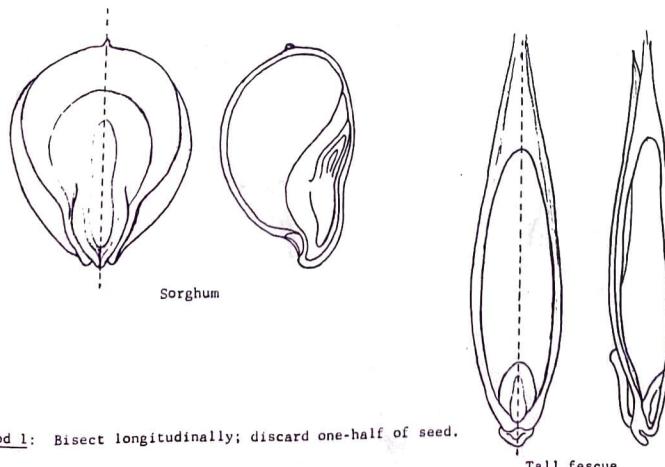
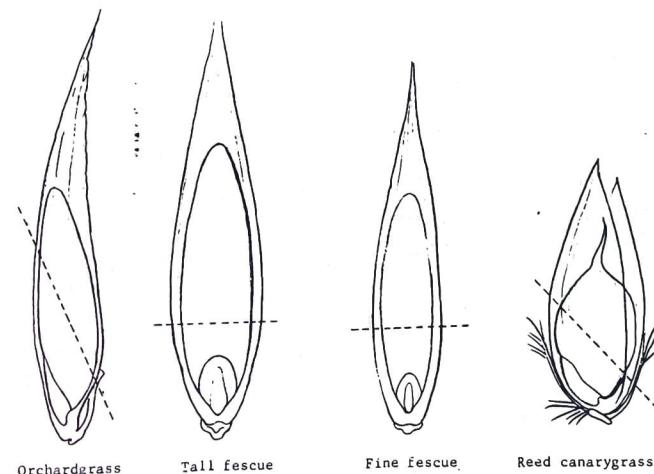


Figure 1. Corn, sorghum, small grains, and large-seeded grasses are prepared for staining by cutting the seeds longitudinally with a razor blade, exposing the main structures of the embryo.

soaking 3 to 4 hours in warm water. Bisect the seeds while still on the blotter, cutting seed longitudinally with one clean sliding cut with a sharp razor blade, exposing the main structures of the embryo. Use one-half of each seed for testing. Immediately transfer the seeds from the blotter to the solution with the razor blade, forceps, or fingers so the seeds do not dry out.

**METHOD 2: Bisect laterally (Figure 2)
(small-seeded grasses)**

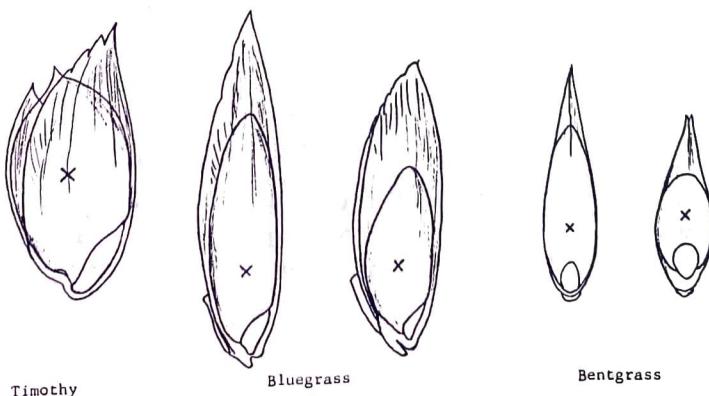
Condition the seeds overnight on top of moist blotters or filter paper. Bisect the seeds while still on the paper, cutting laterally with a sharp razor blade near the center of the caryopsis above the embryo. Place embryo end in tetrazolium solution, transferring the seed with a corner of the razor blade.



Method 2: Section laterally; discard apex portion of seed.

Figure 2. Small-seeded grasses may be prepared for staining by cutting laterally with a razor blade. Only the embryo end is placed in the tetrazolium solution.

PREPARATION OF SEED



Method 3: Pierce with needle.

Figure 3. Small-seeded grasses may be prepared for staining by piercing into the endosperm with a needle.

METHOD 3: Pierce with needle (Figure 3)
(small-seeded grasses)

Condition the seeds overnight on top of moist blotters or filter paper. Puncture the seeds while still on the blotter, piercing through the pericarp into the endosperm near the embryo, but avoid injury to the embryo. Transfer the seeds from the blotter to the solution with the needle tip.

METHOD 4: Remove seedcoat
(Dicots with seedcoats impermeable to tetrazolium)

Condition the seeds overnight in moist rolled towels, between folded blotters, or by soaking 3 to 4 hours in warm water.

Remove seedcoats with forceps, needles, razor blades, thumbnail, or other suitable method. Peanut seedcoats may be removed by lifting up at the pointed end and tearing in a spiral manner. Cotton seedcoats may be removed by slitting the seedcoat longitudinally and spreading it open to free the embryo. Experience and practice will dictate the most appropriate procedures.

Cotton has a thin membrane which adheres to the cotyledons and prevents staining even though the seedcoat is removed. This membrane may be easily removed by a sliding motion between thumb and forefinger after 30 minutes additional soaking in water.

METHOD 5: Conditioning only
(Large-seeded legumes)

Seeds of soybeans and other large-seeded legumes may swell so rapidly and irregularly when placed directly in water or tetrazolium solution that frequently seedcoats burst, cotyledons separate, hypocotyls break, or other damage occurs. It is preferable to condition these seeds slowly in moist paper towels overnight before staining so they absorb moisture slowly without damage to the seed. Staining time may be reduced by puncturing or cutting the seed coats.

METHOD 6: No conditioning or preparation
(Small-seeded legumes)

Seedcoats of these seeds are permeable to tetrazolium and the embryos usually will stain satisfactorily without conditioning. It is necessary merely to place the intact seeds directly in the tetrazolium solution. When the percentage of hard seeds to be expected in the germination test is desired the seeds should be conditioned overnight between wet blotters at 20° C. and stained for 3 or 4 hours at 35° C. If it is desirable to determine the viability of the hard seeds, the seedcoat should be pierced or fractured prior to conditioning.

8. STAINING

Seeds can be stained in a small glass or plastic container, such as a petri dish, Syracuse watch glass, or beaker. Sufficient solution should be used to cover the seeds and to allow for absorption in the case of larger seeds.

As a Rule of Thumb, use a concentrated (1.0%) solution for legumes, cotton, and grasses that are not bisected through the embryo, and a dilute (0.1% to 0.25%) solution for grasses and cereals that are bisected through the embryo.

Suggested staining times are given in Table I for all the crops listed. These times are not absolute, however, and may vary according to the condition of the seed, and concentration and temperature of the tetrazolium solution. As experience is gained, it is possible to make interpretations at an earlier stage of staining. Care should be taken not to overstain seeds as this causes degeneration, making interpretations difficult and uncertain.

9. EVALUATION OF TETRAZOLIUM TESTS

9.1 Preparation for interpretation

When seeds are to be evaluated immediately after staining is completed, siphon off most of the tetrazolium, using a medicine dropper if seeds are small. A small amount of liquid should remain to prevent the seeds from drying out. If seeds are not to be evaluated for several hours, replace the tetrazolium solution with water and place the seeds in cold storage.

Evaluation of small-seeded grasses can be facilitated by applying a lactophenol clearing solution after staining. This solution causes the hulls to become transparent and the stained embryos to be sufficiently visible for evaluation. To clear the seeds, first siphon off the tetrazolium with a medicine dropper, then blot up the remaining liquid with small squares of blotting paper before applying the lactophenol. For 100 seeds of bluegrass, 2-3 drops of lactophenol are usually sufficient. Bluegrass hulls will become transparent in ½ hour, timothy in 10 minutes.

Examine small seeds under a stereoscopic microscope. It is convenient to leave the seeds in the staining dish and make the separations with a spear-pointed needle, teasing needle, or forceps, sliding the seeds into separate groups—germinable and non-germinable. Large seeds may be examined without a microscope, but some magnification is usually helpful.

Seedcoats of legumes must usually be removed before examination. This is best accomplished with use of forceps, teasing needles, or finger nails. Care must be taken to prevent breaking of radicles and other damage to the seeds. Small-seeded legumes such as clovers and alfalfa may be cleared with lactophenol solution and examined over transmitted light without removing the seedcoats.

9.2 General information for evaluating tetrazolium tests

Accurate interpretation of the tetrazolium test is dependent upon:

1. Knowledge of seed and seedling structures and seed germination.
2. Understanding of the mechanism of the test and its limitations.
3. Combining interpretation of staining patterns with other visible aspects of seed quality.
4. Experience with making comparative germination tests.

Normal red color in tetrazolium testing develops when hydrogen from respiration processes of living cells combines with absorbed tetrazolium solution. Sound embryo tissues absorb tetrazolium slowly and tend to develop a lighter color than embryos that are bruised, aged, frozen, or disturbed in other ways. Firm and sound non-red tissues that grade uniformly into stained tissues indicate the lack of tetrazolium penetration rather than presence of death. A gradual reduction of color intensity from the surface to the interior of seed tissue indicates slow absorption of tetrazolium. Distinct color changes between normally stained, firm tissues and white flaccid tissues are evidence that the unstained tissues are dead.

Color is only one of many factors that must be carefully observed when interpreting a test. Turgor of tissues, absence of critically located fractures, bruises, insect cavities, etc., must be noted. Seed parts, as well as the seed as a unit, must serve as the basis for evaluation. It takes only one fracture or one small but deeply seated dead, broken, or missing spot in a vital position, such as the point of attachment of roots and cotyledons, to make a non-germinative seed out of an otherwise sound seed.

Curling and elongation of the root-shoot axes of live embryos of bisected corn, grasses, and small grains serves as a morphological indication of viability. Elongation occurs after standing several hours in liquid because of the initiation of germination processes. The root-shoot axes of non-viable seeds remain flat under similar conditions.

The analyst should be familiar with cell division areas in embryos. In grasses the location of these areas include tips of radicles and seminal roots and the base of plumules. If dead area includes mesocotyl and seminal roots, the embryo cannot develop into a seedling. In wheat and rye the tips of coleorhizas are frequently injured and dead. In corn that has been shelled at high moisture, the upper and lower tips of the scutellum frequently fail to stain. These symptoms do not necessarily prevent germination under favorable conditions, especially if seed are properly treated with a suitable fungicide.

In legumes and other dicotyledonous seeds early cell division occurs largely in the radicle and plumule.

Fractures and bruises in legume seed occur frequently in hypocotyls and at points of attachment of cotyledons and hypocotyls. Injuries that

involve growing points or that are located between or adjacent to essential structures, are more critical than injuries of similar magnitude but in more remote regions as in tips of cotyledons.

Many seeds are neither completely alive nor completely dead. A knowledge of the relationship of seed structures to seedling structures is necessary to interpret the importance of unstained seed tissues. For example, unstained radicle tips are generally interpreted differently in grasses than in legumes—most legumes have a tap root system, and if the radicle does not grow no tap root system develops; however, most grasses have seminal root buds in the embryo which can develop and produce a normal seedling even if the radicle does not grow. Seed and seedling structures of corn and beans are shown in Figures 4 and 5.

In making interpretations, staining patterns should be correlated with the seedling descriptions included in the Appendix to the Rules for Testing Seeds.

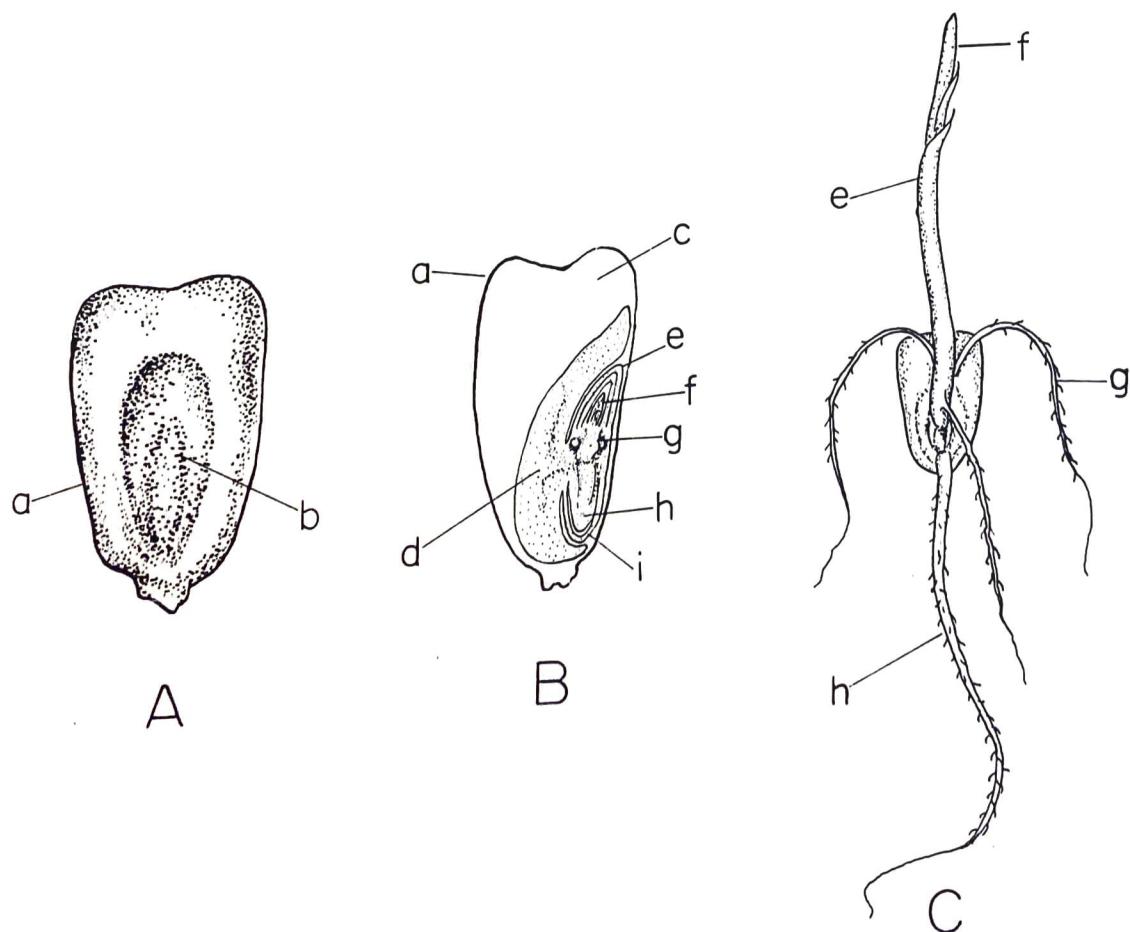


Figure 4. Seed and seedling structure in the grasses (corn). A. External view of caryopsis. B. Bisectional view of caryopsis. C. Seedling. a, pericarp; b, embryo; c, endosperm; d, scutellum; e, coleoptile; f, plumule; g, seminal root; h, radicle; i, coleorhiza.

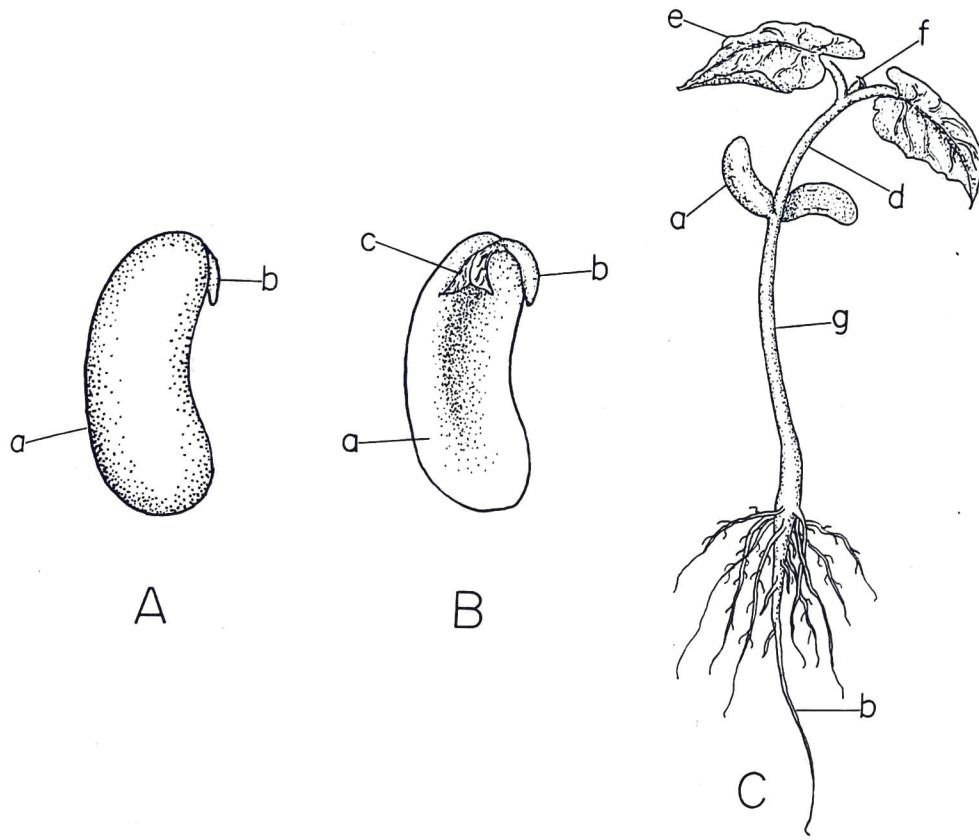


Figure 5. Seed and seedling structure in dicotyledonous species (beans). A. External view, seed coat removed. B. Interior view, one cotyledon removed. C. Seedling. a, cotyledon; b, radicle; c, plumule; d, epicotyl; e, primary leaf; f, growing point; g, hypocotyl.

If deterioration is of recent occurrence, bruised tissues tend to develop a dark red color indicating rapid penetration of tetrazolium and a rapid respiration rate.

Complete or partial fractures may occur with or without bruised tissues, depending upon the dryness of the seed at time of injury. Fractures also occur when dry, brittle seed of numerous crops absorb water too rapidly or when seed coats are being removed. Surfaces of fractures that occur prior to staining appear whitish or sometimes dark red. Those caused by the analyst at time of seed coat removal usually appear normal red and fully turgid. Since healing can occur, especially in hypocotyls in bean seedlings, partial fractures of roots do not necessarily indicate non-germinable seed.

Aged tissues tend to appear flaccid and to develop weak or mottled color presenting an appearance that is intermediate between that of strong live tissues and completely dead tissue. Tissues of intermediate stages of aging tend to remain white near cut surfaces with deep red to nearly normal red color in deeper-seated tissues. The depth of non-colored tissues resulting from cut surface provides a good indication of the relative stage of

embryo deterioration. In seeds that are stained without cutting, mottling of non-cut surface areas indicates areas of advanced tissue deterioration.

9.3 Accuracy of results

The tetrazolium percentage is the percentage germination to be expected when the lot is germinated under very favorable conditions. Favorable germinating conditions include the proper use of fungicide for seed lots that are susceptible to mold infection in germination tests. Cotton, peanuts, soybeans, and lima beans are examples of crops that are benefited by fungicide treatment, particularly when aged or injured.

Properly conducted tetrazolium and germination test results are generally in close agreement, within the range of normal sampling variation. Differences of 3-5% may be due entirely to unavoidable sampling error. Differences in results are usually smaller with high quality seed than with low quality seed, with large-seeded crops than with small-seeded crops, and with uniform seed lots than with non-uniform lots.

Large discrepancies between tetrazolium and germination test results do not necessarily mean that the tetrazolium test is in error. Retests by both methods should be made to establish the cause of differences.

Discrepancies between tetrazolium and germination tests results may be due to several reasons. The following checklist of causes is presented to assist in making a systematic rundown of the reasons for these discrepancies:

- (a) Sample differences. Wide variations due to sampling are especially frequent in chaffy grasses, range grasses with many immature seeds, mechanically injured and hulled lots, and blended lots.
- (b) Improper germination testing techniques. Improper moisture, light, and temperature, or other conditions may result in erratic germination of seed lots especially sensitive to these factors.
- (c) Improper tetrazolium testing techniques. There can be considerable latitude in methods of staining, but interpretation of results is much more critical. Repeated comparisons of tetrazolium and germination results are often needed to help in making necessary adjustments in evaluating.
- (d) Dormant seed. The tetrazolium test does not differentiate between dormant seed and non-dormant seeds. Thus, in those kinds of seed with deep-seated dormancy, tetrazolium test results are considerably higher than germination test results. Tetrazolium test results should closely approximate the total of germination plus dormant seed percentages.
- (e) Hard seeds. Hard seed content may vary between the two tests, but the total tetrazolium plus hard seed percentage should approximate the total germination plus hard seed percentage. To over-

come to a certain extent the discrepancy in hard seed content, condition the seeds for a longer period of time at a cool temperature.

- (f) Seed borne organisms. In germination tests on low quality seed, fungus infection may prevent all viable seeds from germinating normally and the germination percentage will be too low. Agreement between the two tests is often better if the seeds are treated with a fungicide before the germination test.
- (g) Chemical injury. Fumigation injury and overtreatment with mercurial seed treatments may not be detected with the tetrazolium test. The chemical damage that prevents normal germination may not inhibit the tetrazolium staining process.

9.4 Evaluation groups

For interpretation purposes, seeds of most crop species fall into one of five groups based on structure and method of preparation. These are (a) corn, sorghum, small grains; (b) large-seeded grasses; (c) small-seeded grasses; (d) legumes; and (e) dicots other than legumes. Familiarity with these five basic seed groups enables the analyst to evaluate any kind of crop seed, with but minor modifications needed for a few kinds. General procedures for interpreting tetrazolium tests for seeds in each of these groups are detailed below:

GROUP A: Corn, sorghum, small grains (Figure 6 and Plates I and II)

Magnification of about 5-7X is desirable. Since the seeds are large, the various embryo structures are clearly visible, and distinct staining patterns occur.

A normal tetrazolium stain appears cherry-red to the naked eye. Under magnification, the scutellum appears to have many dark red dots against a light red background.

-
- Figure 6. Criteria for interpreting tetrazolium test results on wheat seed. Black areas indicate stained, living tissue; white areas represent unstained and dead tissue.
- No. 1 GERMINABLE. Entire embryo stained bright red.
- Nos. 2-5 GERMINABLE. Extremities of scutellum unstained.
- No. 6 GERMINABLE. Extremities of scutellum, radicle tip, and coleorhiza unstained.
- No. 7 NON-GERMINABLE. More than three-fourths of radicle unstained.
- No. 8 NON-GERMINABLE. Plumule unstained.
- No. 9 NON-GERMINABLE. Central portion of scutellum and scutellar node unstained.
- No. 10 NON-GERMINABLE. Embryonic axis unstained.
- No. 11 NON-GERMINABLE. Extremities of scutellum and plumule tip unstained.
- No. 12 NON-GERMINABLE. Entire upper half of embryo unstained.
- No. 13 NON-GERMINABLE. Scutellum unstained.
- No. 14 NON-GERMINABLE. Scutellum, radicle, and coleorhiza unstained.
- No. 15 NON-GERMINABLE. Stain very faint pink.
- No. 16 NON-GERMINABLE. Entire embryo unstained.

