TETRAZOLIUM AS AN INDICATOR OF VIABILITY OF CERTAIN GRASS SEEDS

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GRASS SEEDS

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INTRODUCTION

For many years seedsmen and seed analysts have recognized the need for a "quick test" for determining seed viability. Purity and noxious weed seed examinations can be completed within a few hours, but germination tests require from one week to a month, or even longer.

The use of 2,3,5-triphenyl tetrazolium chloride as an indicator of the viability of seeds has received considerable interest in the past few years. A number of experiments have produced results which show a good correlation between tetrazolium staining and germination tests. Whereas ordinary germination procedures require several days, determinations can be obtained in a few hours by the tetrazolium method.

Results of the tests are based on the color change produced by the reaction of tetrazolium salt in aqueous solution with the tissue of the living embryo of the seed. On the addition of a hydrogen radical, the colorless tetrazolium compound is reduced to a pink or crimson formazan compound. If embryos are killed by boiling in water for a few minutes, no color change occurs when placed in the tetrazolium solution. This makes it apparent that the reaction is dependent on respiratory processes in the embryo.

This experiment was conducted for the purpose of evaluating the use of 2,3,5-triphenyl tetrazolium chloride as a test for quick viability determination of certain grass seeds.

REVIEW OF LITERATURE

The first seed viability tests with tetrazolium salts were conducted by Lakon (11)[1] in Germany. He tested several salts and found 2,3,5-triphenyl tetrazolium chloride to be most suitable. With wheat, rye and barley his procedure consisted of excising the embryo; with oats the embryo end of the grain was clipped; with corn the seeds were soaked overnight and bisected longitudinally. Embryos or seed parts to be tested were immersed in a 1% tetrazolium solution and placed in the dark. After 4 to 24 hours, living parts of the embryo stained bright red. Lakon stated that the tetrazolium method, in contrast to the germination tests, practically eliminates experimental errors, and that data from two tests of 100 kernels each, treated in this manner have been found to provide the same accuracy as direct germination experiments carried out as four tests of 100 grains each.

Porter, Durrell and Romm (16) employed Lakon's methods with several different agricultural seeds. They found close agreement between staining and germination tests in 13 samples of corn, 2 each of wheat and oats, 3 samples each of barley and rice, and one each of buckwheat, popcorn, soybeans, peas, and Bahia grass. Comparisons for vetch,

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[1] Numbers in parenthesis refer to Literature Cited.

sorgo, 2 samples of oats and peas, and one of barley were not in close agreement, but differences were not too great.

Slightly modifying Lakon's methods with cereal seeds, Cottrell (5) reported high correlation with standard germination tests. Seeds soaked overnight were bisected symetrically and just covered with 1% tetrazolium salt for 4 hours in the dark. In cereals other than maize, ability to germinate was indicated by staining at least half of the scutellum, the whole shoot, and junction between root and shoot. In maize the entire scutellum must be stained.

Shuel (18) attempted to eliminate the necessity for bisecting the seeds. He conducted tests with barley, oats and wheat. The entire grain was immersed in a 1% solution of tetrazolium chloride and evaluation of staining was made after 5 hours by external observation of the embryo. He reported that the tests appeared to give a quick, reliable index of germination with new seeds. For old seeds with viability less than 60%, tetrazolium tests were much less accurate.

Flemion and Poole (6) worked with seeds representing 17 families and 58 species, primarily woody plants, with embryos ranging from non-dormant to dormant. All tests were carried out with excised embryos. Germination capacity was determined by the rapid viability test, which consisted of placing excised embryos on moist filter paper in Petri dishes at room temperature and observing for 2 to 7 days. For the tetrazolium test, embryos were placed in vials, covered with

excess tetrazolium and placed in the dark for 24 hours at 68° F. Significant correlation between the two methods was obtained, but large and frequent deviations were obtained in many individual tests.

Goodsell (8) used tetrazolium chloride in an attempt to determine frost injury in seed corn. When tetrazolium tests were made immediately after freezing, poor correlation was obtained. If the corn was dried to a moisture level of 12% or less before testing, a high correlation was found between tetrazolium readings and germination percentages. Tests were made with a 0.25% tetrazolium solution to which 1 cc of 10% NaOH per 100 cc of solution was added.

Bennett and Loomis (3) encountered the same difficulty as Goodsell in a similar study. Using a 0.05% solution, they were able to estimate freezing injury fairly accurately.

Gadd (7), in connection with a review of biochemical tests for seed germination, concluded that such methods were useful for special purposes, but should not replace germination tests as advocated by Lakon.

Brewer (4) employed tetrazolium methods in a study to determine damage in artifically cured peanuts. The separated embryos were held in warm water (115° F) for an hour, then immersed in a 2% solution of tetrazolium and incubated in the dark at 115° F for another hour, after which readings were made. The degree of damage was interpreted according to shades of red staining obtained. Tetrazolium and

germination tests showed essentially the same degree of damage.

Jensen, Sachs and Baldauski (10) investigated several enzyme systems, prepared from corn embryos, as to their ability to reduce the tetrazolium reagent. They concluded that the presence of active enzyme systems does not necessarily indicate seed viability, but the absence of active dehydrogenases probably indicates loss of germinating ability.

Smith and Throneberry (19) investigated the mechanism of the tetrazolium reaction in seeds. They concluded that the dehydrogenases are responsible for tetrazolium reduction and since the dehydrogenase enzymes constitute delicate and complex systems vital in respiratory activity it might be presumed that an accurate assay of dehydrogenase activity would also be a good measure of viability.

Isely (9) experimented with oats, wheat, rye and barley. His methods consisted of pre-soaking seeds 3 to 4 hours, hulling (oats), bisecting longitudionally, and placing in a 0.05% solution of tetrazolium. Readings were made in 3 to 4 hours, the live embryos being differentiated from dead by red staining. He concluded that under most conditions reasonably accurate viability tests can be made with tetrazolium and that, in general, the tests are quite precise for high viability seeds and less so or occasionally erratic on poor seed.

Using certain coniferous seeds, On (14) found close comparison with tetrazolium staining and viability determined

by germination tests.

Close agreement between tetrazolium tests and standard germination tests was found by Lambou (12) in 16 out of 17 lots of cotton seed. For the staining tests he immersed longitudional sections of seed, with lint and coat removed, in a 2% tetrazolium solution for 4 hours.

Parker (15), conducting tests with conifer seeds, found that tetrazolium staining seemed to overrate germination percentage if seed had a period of dormancy. He obtained good results if staining tests were made on a sample of seed as they arrived from the seed house before stratification, and comparing these with percentages of germinating seed after stratification in moist cloth at 40° F for two months.

Roistacher, Bald and Baker (17) developed and tested a standard procedure for testing dormancy and germinability of Gladiolus cormels. The tetrazolium tests gave reasonably consistant estimates in nearly all lots tested.

Oberle and Watson (13) tested samples of pollen of peach, pear, apple and grape, using a 1.5% solution of tetrazolium in a 1% agar and 10% sucrose medium. Results indicated pollen grains of these fruits may stain although not capable of germination. They concluded that tetrazolium is of no value as an indicator of pollen germination for the fruits tested.

Tetrazolium tests on Kentucky bluegrass seeds carried out by Bass (2) were found to be reasonably reliable. The

seeds were bisected laterally and the basal portions soaked in a 0.05% solution of tetrazolium for 24 to 48 hours. The lemma and palea were subsequently rendered transparent with lactophenol so that the embryo was visible for examination. All separations of viable and non-viable seeds were made with the aid of a binocular microscope. He examined 318 samples with an average difference of about 5% between the tetrazolium and germination tests.

STRUCTURE OF SEEDS STUDIED

In order to properly apply the tetrazolium procedure it is necessary to have a general knowledge of the structure of the seeds involved.

Seeds of sand bluestem, Andropogon hallii Hack., sideoats grama, Bouteloua curtipendula (Michx.) Torr., smooth brome, Bromis inermis Leyss, switch grass, Panicum virgatum L. and sand lovegrass, Eragrostis trichodes (Nutt.) Nash were used in this study.

Commercial seeds of sand bluestem, smooth brome, switch grass and sideoats grama are generally unhulled, but all tetrazolium treatments in this experiment were made with seeds free of the lemma and palea; therefore, structure of such seeds are considered here.

Although structure of the grass seed varies considerably among the various species, the following general characteristics apply to seeds used in this study.

The major components of the grass caryopsis are the seed coat, endosperm and embryo. With the exception of sideoats grama, the greater portion of the volume of the seed is made up of the endosperm. The embyro is located at the base of the seed on the dorsal side and its outline is visible through the outer seed covering.

The front and lower portion of the embryo consists of

the embryonic stem and root systems, with the root initial extending toward the base of the seed and the shoot initial extending toward the tip. The scutellum lies behind and above this portion of the embryo and is in contact with the endospern on one side. It partially surrounds and is attached to the embryo shoot system at the first node.

The sand bluestem grain is narrowly elliptical in shape, 4 to 6 mm in length, with the embryo occupying slightly less than half the face area when viewed dorsally.

In dorsal view the sideoats grama seed is oblong in shape and in side view it is flat and straight. It is $2\frac{1}{2}$ to 3 mm in length. The scutellum of this seed is relatively large, so that the embryo makes up approximately three-fourths of the volume of the grain.

The smooth brome seed is very thin and flat, narrowly oblanceolate in shape, 6 to 8 mm in length and 1 to $1\frac{1}{2}$ mm in width. The embryo is very small, being generally less than 1 mm in length or width.

The switch grass grain is angularly elliptical in shape, $1\frac{1}{2}$ to 2 mm in length, with the embryo extending over more than half the face of the seed when viewed dorsally.

The sand lovegrass seed is oval in shape with a deep longitudional cavity on the ventral side. It is 1 to $1\frac{1}{2}$ mm in length and 1 mm or less in width. The embryo covers approximately half the face of the seed in dorsal view.

METHODS

A total of eight lots of seeds were employed in a study of viability determined by tetrazolium staining methods as compared to actual germination.

One lot each of sand bluestem, sideoats grama, switch grass and smooth brome was secured from seed harvested in 1953. One lot each of sand bluestem, sideoats grama and sand lovegrass was secured from seed harvested in 1950. One lot of smooth brome was secured from seed harvested in 1951.

Tetrazolium tests were made with hulled (lemma and palea removed) entire seeds. Preliminary examinations with unhulled switch grass and smooth brome seeds proved unsatisfactory. Four hundred (4 x 100) seeds were employed for each staining test.

Preliminary tests were made with tetrazolium solutions of 0.05%, 0.1%, 0.25%, 0.5% and 1% strength. A 0.25% solution was used for all tests on which data are presented. Weaker solutions effected staining, but required a longer period of treatment. Stronger solutions did not appreciably speed up the reaction, nor did they effect darker staining. The stock solution was kept in the dark when not in use.

Tetrazolium treatment was carried out in small vials, one hundred seeds to a vial. The seeds were covered with

an excess amount of solution and vials placed in the dark at room temperature. Readings for all seeds except sand bluestem were made after four hours of treatment. Sand bluestem required six hours in the solution for staining to be accomplished. Most of the determinations were made without magnification, but some required the use of a 10X hand lens. Readings were made immediately after removal from the solution. Drying of the seeds made readings more difficult. When drying occured seeds were placed in a Petri dish with just enough water to keep them moist until determinations were completed.

Preliminary investigations revealed considerable variation in the amount and intensity of staining among the five species studied. Therefore, the method of interpretation of tetrazolium staining varied among the different species.

While a great deal of time was spent in arriving at the following methods, proper interpretation of tetrazolium staining requires a degree of skill which can only be acquired through a more extensive study and experience.

Sand bluestem and sideoats grama seeds were judged to be viable if at least 50% of the embryo showed a bright red staining. Occasionally a large percent of the scutellum was stained a bright red with the embryonic root and/or shoot showing little or no staining. Based on preliminary staining tests comparisons with actual germination percentages, these seeds were considered viable.

In the determination of viability of switch grass seeds, intense staining of the scutellum did not appear

to be of major importance. Seeds were considered viable if the embryonic axis (embryo minus the scutellum) was stained a bright red color. Although the scutellum was stained in most of the viable seeds, in general the intensity of staining was considerably less than in the reminder of the embryo.

Seeds of smooth brome and sand lovegrass were considered viable if the entire embryo was stained. Intensity of staining did not appear to be a determining factor. All readings of brome and lovegrass seeds were made with the aid of a 10X hand lens.

Germination tests were carried out in accordance with procedures prescribed by the Association of Official Seed Analysts (1). Four hundred (4x 100) seeds were employed in each germination test.

RESULTS AND DISCUSSION

Results of the tests conducted are presented in Tables 1 and 2. Table 1 gives the germination and tetrazolium staining percentages of each sample. Percentages for each lot of seed are given in Table 2.

Germination of individual lots ranged from 1.25% to 88.75%, representing viability over a wide range of levels. Lot number eight showed a difference of 8.25% between germination and tetrazolium staining; while differences between the two methods in the remaining lots ranged from 1% to less than 4%.

Statistical analyses of the results were made according to the methods outlined by Snedecor (20). The analysis of variance of each lot is presented in Table 3 and the analysis of the entire experiment is given in Table 4.

Lot number eight showed a significant difference between germination and staining results at the 5% level. This lot consisted of sand lovegrass with some hard seeds present, which may partially explain this difference. The remaining lots and the experiment as a whole indicated a close agreement between the two methods of determining viability.

Table 1. Germination and Tetrazolium Staining Percentages of Samples.

Lot	Percent Germinating				Percent Staining with Tetrazolium			
	Sample				Sample			
	1	2	3	4	1	2	3	4
I	36	37	49	45	41	47	46	48
II	52	66	59	66	55	64	58	60
III	46	35	34	35	45	34	38	39
IV	87	89	93	86	93	91	94	90
V	0	1	2	2	7	4	0	0
VI	66	70	74	74	71	76	67	74
VII	57	59	71	57	63	54	60	55
VIII	21	18	12	13	19	27	28	23

Table 2. Germination and Tetrazolium Staining Percentages of Lots.

Lot	Percent Germinating	Percent Staining with Tetrazolium
I	41.75	45.50
II	60.75	59.25
III	37.50	39.00
IV	88.75	92.00
V	1.25	2.75
VI	71.00	72.00
VII	61.00	58.00
VIII	16.00	24.25

Table 3. Analyses of Variance of Lots.

Lot	Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
I	Methods	1	28	28
	Error	6	148	24.7
II	Methods	1	4.5	4.5
	Error	6	177.5	29.6
III	Methods	1	5.0	5.0
	Error	6	158.5	26.4
IV	Methods	1	21.25	21.25
	Error	6	38.75	6.44
V	Methods	1	4.5	4.5
	Error	6	37.5	6.25
VI	Methods	1	2	2
	Error	6	90	15
VII	Methods	1	18	18
	Error	6	190	31.7
VIII	Methods	1	136	136*
	Error	6	105	17.5

*Exceeds significance at the 5% level.

Table 4. Analysis of Variance of Entire Experiment.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Methods	1	54.3	54.3
Error	62	46158.0	774.5

SUMMARY AND CONCLUSIONS

This study was concerned with the evaluation of the use of 2,3,5-triphenyl tetrazolium chloride as a test for quick determination of viability of certain grass seeds.

Samples from eight lots of seeds, including sand bluestem, sideoats grama, smooth brome, switch grass and sand lovegrass were used in a study of the comparisons between viability as determined by tetrazolium staining and actual germination tests.

Methods involved soaking hulled entire seeds in a 0.25% tetrazolium solution for four to six hours, after which readings were made and viability determined according to the amount and intensity of staining.

The data presented indicate that a reasonably accurate estimate of germinability may be made by the tetrazolium method.

The principal advantage of the tetrazolium test is its speed, plus the fact that a relatively small amount of equipment is needed. However, if accurate results are to be obtained, a certain degree of skill and experience is required. Special techniques must be worked out for each kind of seed.

LITERATURE CITED

1. Association of Official Seed Analysts. Rules for testing seeds. Proc. Assoc. Off. Seed Anal. 39:34-45. 1949.

2. Bass, Louis N. 2,3,5-triphenyl tetrazolium chloride as an indicator of the viability of Kentucky bluegrass seed. Proc. Assoc. Off. Seed Anal. 43:131-135. 1953.

3. Bennett, Norah and Loomis, W. E. Tetrazolium chloride as a test reagent for freezing injury of seed corn. Plant Physiol. 24:162-174. 1949.

4. Brewer, H. E. Tetrazolium chloride as a test for damage in artifically cured peanuts. Science 110:451-452. 1949.

5. Cottrell, H. J. Tetrazolium salt as a seed germination indicator. Nature 159:748. 1947.

6. Flemion, Florence and Poole, Harriet. Seed viability tests with 2,3,5-triphenyl tetrazolium chloride. Contr. Boyce Thomp. Inst. 15:243-258. 1948.

7. Gadd, Ivor. Biochemical tests for seed germination. Proc. Int. Seed Test. Assoc. 16:235-253. 1950.

8. Goodsell, S. F. Triphenyl tetrazolium chloride for viability determination of frozen seed corn. Jour. Amer. Soc. Agron. 40:432-442. 1948.

9. Isely, Duane. Employment of tetrazolium chloride for determining viability of small grain seeds. Proc. Assoc. Off. Seed Anal. 42:143-153. 1952.

10. Jensen, C. O., Sachs, W. and Faldauski, F. A. The reduction of triphenyl tetrazolium chloride by dehydrogenases of corn embryos. Science 113:65-66. 1951.

11. Lakon, G. The topographical tetrazolium method for determining the germinating capacity of seeds. Plant Physiol. 24:389-394. 1949.

12. Lambou, M. G. 2,3,5-triphenyl tetrazolium chloride as a rapid indicator of viability in cotton seed. Science 117:690-693. 1953.

13. Oberle, G. D. and Watson, R. The use of 2,3,5-triphenyl tetrazolium chloride in viability tests of fruit pollens. Amer. Soc. Hort. Sci. Proc. 61:299-303. 1953.

14. On, D. 2,3,5-triphenyl tetrazolium chloride as a viability indicator of certain coniferous seeds. Jour. Forestry 50:868. 1952.

15. Parker, J. Some applications and limitations of tetrazolium chloride. Science 118:77-79. 1953.

16. Porter, R. H., Durrell, M. and Romm, H. J. The use of 2,3,5-triphenyl tetrazolium chloride as a measure of seed germinability. Plant Physiol. 22:149-159. 1947.

17. Roistacher, C. N., Bald, J. G. and Baker, K. F. The tetrazolium test for dormancy and germinability of Gladiolus cormels. Science 118:186-187. 1953.

18. Shuel, R. W. Seed germinability tests with 2,3,5-triphenyl tetrazolium chloride. Sci. Agri. 28:34-38. 1948.

19. Smith, F. G. and Throneberry, G. O. The tetrazolium test and seed viability. Proc. Assoc. Off. Seed Anal. 41:105-109. 1951.

20. Snedecor, G. W. Statistical Methods. Ames, Iowa: The Iowa State College Press. 4th ed. 214-227. 1946.

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