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The Relationship Between Tne Tetrazolium Test, Soil Cold Test, and Standard germination test in assessing sorghum [Sorghium Bicolor(L.) Moench] Seed Quality

H. C. Potts

C. C. Baskin

James C. Delouche

THE RELATIONSHIP BETWEEN THE TETRAZOLIUM TEST, SOIL COLD TEST, AND STANDARD GERMINATION TEST IN ASSESSING SORGHUM [*SORGHUM BICOLOR*(L.) MOENCH] SEED QUALITY[1]

S. Paliwal, C.C. Baskin, and J.C. Delouche[2]

ABSTRACT

Tetrazolium tests, soil cold tests, and standard germination tests were conducted on 40 lots of grain sorghum [*Sorghum bicolor* (L.) Moench]. Based on tetrazolium evaluations, seeds were grouped into high vigor, medium vigor, low vigor, and non-germinable categories. The high plus medium vigor categories of tetrazolium were significantly correlated at the 0.01% level of probability with emergence in soil cold test (r=0.936). The high plus medium plus low vigor categories were significantly correlated at the 0.01% level of probability with standard germination (r=0.900). These results demonstrate the potential of the tetrazolium test for assessing seed quality in sorghum.

Additional index words: **Germination, Vigor testing, Seed quality evaluation.**

INTRODUCTION

The standard germination test is the most widely used seed quality test. However, standard germination does not predict field emergence under a wide range of conditions. The soil cold test is the most widely used vigor test for corn. It measures the ability of seed to survive a period of cold, moist conditions after planting.

The tetrazolium test can be used to estimate vigor as well as viability. Moore (1964) suggested that the tetrazolium test provides a straight forward measure of seed soundness and not an interaction of seeds and planting conditions. Delouche et al. (1962) suggested that the tetrazolium test for determining seed viability provides a much more reliable basis for decisions regarding seed viability than intuition or experience, and results of the test are available in hours rather than days or weeks. Moore and Goodsell (1965) used the tetrazolium test for predicting cold test performance of corn seed lots. They obtained a correlation of 0.96 between tetrazolium 1-3 categories (their tetrazolium evaluation included eight categories) and cold test emergence of 92 commercial corn seed lots. Tunstul (1974) reported correlations of 0.82 and 0.66 between standard germination and estimated germination, both significant at the 0.01% level of probability, using tetrazolium to evaluate 50 lots of grain sorghum in each of two different testing periods.

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[1] Approved for publication as Journal Article No. J-7416 of the Mississippi Agricultural and Forestry Experiment Station, Mississippi State University.

[2] Post Doctorate Trainee, and Professors of Agronomy, respectively, Mississippi State University, Miss. State, MS. 39762.

The use of the tetrazolium test for estimating vigor of soybean, cotton, corn and wheat seed is discussed in the Seed Vigor Testing Handbook of the Association of Official Seed Analysts (1983).

The successful use of tetrazolium tests in predicting standard germination and cold test performance, i.e. seed quality and potential performance, should provide a rapid method of evaluation of sorghum seed.

MATERIALS AND METHODS

Seed Lots

Seeds of 37 lots of commercial sorghum hybrids were obtained from Asgrow Seed Company, Cargill Hybrid Seeds, and Funk Seed's International in 1986 and 1987. Lots were selected by the respective companies to represent a range in seed quality based on company tests. In addition, seed of three "B" lines were obtained from Funk Seed's International in 1986. All 40 seed lots were stored at 10°C ad 50% relative humidity during the period that tests were being conducted.

Seed Quality Tests

Standard germination tests were conducted and evaluated according to the Association of Official Seed Analysts (AOSA) "Rules for testing seeds" (1985). Soil cold tests were conducted at 10°C for 7 d using a mixture of 50% screened field soil and 50% builders sand. One thousand g of sand-soil mixture were placed in a 20x27x9.5cm plastic crisper. After leveling and firming the sand-soil mixture, four replications of 50 seeds each were planted (two replications per crisper), then covered with an additional 1000 g of sand-soil mix. The sand-soil mix was again leveled and firmed and enough water (pre-chilled to 10°C) was added to each crisper to bring soil moisture content to 60% of saturation. The crispers were covered and placed in a chamber at 10°C for 168 ± 1 h, then transferred to 25°C for 120 ± 1 h. Emerged, normal seedlings were counted.

Two replications of 50 seeds each from each sorghum lot were used to evaluate seed lots based on tetrazolium staining patterns. Seeds were pre-conditioned in moistened seed germination towels for 12 to 16 h at a constant 25°C. After pre-conditioning, seeds were cut longitudinally with a sharp razor blade as nearly as possible through the center of the embryo such that both plumule and radicle would be visible in evaluation. Seeds were not completely bisected so that both halves of the seed would be together and the evaluation made using both halves of the seed. After cutting, seeds were immediately placed in water until the entire sample was cut, then transferred to a 0.5% aqueous solution of 2,3,5 - triphenyl tetrazolium chloride and permitted to stain for approximately 1 h in darkness at 40°C. The tetrazolium solution was decanted and the seeds rinsed in cool tap water three times. Seeds were covered with water and placed in a refrigerator at approximately 5°C until they could be evaluated. The tetrazolium evaluations were conducted as soon as possible after staining. The time lapse between staining and evaluation was never more than 24 h. Evaluation of staining patterns of the embryos was made under a magnification of 10 or 20x as needed. Each seed was evaluated based on staining pattern, intensity of stain, and location of stained or unstained embryonic tissues. Based on these criteria, seeds were divided into four categories: high vigor, medium vigor, low vigor, and non-germinable.

The high vigor category included seeds which had no dead or weakly stained tissue in the embryonic axis or scutellum. The embryonic axis was uniformly stained a dark pink to light red. The scutellum was uniformly stained generally lighter than the embryonic axis usually with dark red specks or flecks; or the scutellum, particularly the ends were stained lighter than the middle part. The scutellum may have had a yellowish or greenish yellow color in these lighter stained areas through a very light pink stain. The dark flecking may still have been evident.

The medium vigor category included seeds which had the embryonic axis and/or scutellum uniformly stained but slightly darker than in the high vigor category. The slightly darker stain was particularly evident in the scutellum area. The dark flecking may or may not have been present and was usually less than in the scuttelar tissue of the high vigor seeds. The very tips of the scutellum may have been unstained.

The low vigor category included seeds which had very darkly stained embryonic tissue. The embryonic axis may or may not have been stained darker than the scutellum. The intensity of the stain may have appeared almost a dark purple or the embryo may have been very weakly stained, a very light pink with no yellowish or greenish yellow background color in the scutellar tissue. There was an absence of dark flecking in weak tissue. The ends of the scutellum may have been unstained as long as the area of the attachment of the embryonic axis to the scutellum was live, functional tissue.

The non-germinable category included seeds which did not stain or had parts of the embryonic axis, coleoptile and/or radicle completely unstained. Also if a large portion of the scutellum area was unstained, particularly the area connecting to the embryonic axis completely unstained or the embryonic axis and/or scutellum very weakly stained or milky in appearance or very darkly stained almost blackish in color, the seeds were also considered non-germinable.

In border-line cases between low vigor and non-germinable categories, endosperm soundness was also considered to help decide the category placement. If the endosperm tissue was firm, seeds were categorized as low vigor seeds, whereas seeds containing flaccid tissue in the endosperm were categorized as non-germinable.

The number of seeds in high vigor plus medium vigor categories were considered the strong or vigorous seeds in the lot. Strong or vigorous seeds would be expected to emerge under a wide range of field conditions. Totaling the number of seeds in high vigor, medium vigor, and low vigor categories would correspond to the standard germination percentage.

Experimental Design and Statistical Analyses

The experimental design for all seed quality tests was a completely randomized design. The mean for all replicates of the tetrazolium evaluations for each seed lot were compared with standard germination percentages and with soil cold tests emergence using simple linear regression analyses.

RESULTS AND DISCUSSION

Tetrazolium Evaluation

There were some color variations in embryo staining that appeared to be related to cultivar differences. Embryos of some seed lots stained a bright pink to bright red while others exhibited a brownish red stain. There was apparently no difference in seed quality between the two color variations and once this was recognized, the color variations between lots presented no problem in evaluation.

Tetrazolium Evaluation and Standard Germination

Standard germination of the 40 seed lots ranged from 63.5 to 99.5%. Tetrazolium evaluation estimate of viability for the 40 seed lots ranged from 65.0% to 99.0%. The coefficient of correlation between tetrazolium estimated viability and actual standard germinaiton was 0.900 and was significant at the 0.01% level of probability (Table 1). The difference between observed and predicted values was less than 10% for most seed lots.

Table 1. Coefficients of correlation of the tetrazolium viability with standard germination and vigor evaluation with soil cold test emergence of 40 lots of grain sorghum seed using the tetrazolium test.

	Standard Germination	Cold Test Emergence
Tetrazolium Viability	0.900**	—
TZ Vigor Rating	—	0.936**

**Denotes significance at the 0.01% level of probability.

Tetrazolium Evaluation and Cold Test Performance

Emergence of the 40 seed lots in the soil cold test ranged from 52.5 to 96.0%. tetrazolium high plus medium vigor categories ranged from 39.0 to 97.0%. The coefficient of correlation between tetrazolium high plus medium vigor seed and cold test emergence was 0.936 and was significant at the 0.01% level of probability (Table 1). The difference between observed and predicted values was less than 5% for most lots and did not exceed 10% for any lot.

This method of placing seed into high, medium, low vigor and non-germinable categories based on staining patterns and intensities in the tetrazolium test is an alternative to more elaborate category schemes. It also demonstrates the potential of the tetrazolium test for assessing sorghum seed quality.

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