THE GADA TEST FOR SEED STORABILITY

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The GADA (glutamic acid decarboxylase activity) test has been developed as a sensitive test to measure seed deterioration in storage and to predict relative storability of seed lots. In laboratory tests, GADA has shown good correlation with longevity of corn seed in storage and with seedling vigor of corn and oats (4, 5)[2] and the test is now ready for field testing under commercial conditions. These instructions have been prepared for those who want to evaluate its use in seed quality control programs.

Background

As seeds age in storage, a number of deteriorative changes occur before they die. Many of these changes can be measured in the laboratory. One of the early changes that can be detected is lower activity of enzymes, including glutamic acid decarboxylase. Grain storage research (1, 2, 3, 6, 7, 8) has associated glutamic acid decarboxylase activity (GADA) with viability and milling quality in stored wheat, corn, and rice grain. This work served as the basis for development of the GADA test for seeds.

The test is performed by adding glutamic acid to finely ground seed. The glutamic acid is broken down by the decarboxylase enzyme present in the seed, giving off carbon dioxide as one of the breakdown products. Measurement of the carbon dioxide evolved is an indication of the amount of decarboxylase enzyme activity in the seed. Other factors being equal, the lower the GADA, the greater the amount of seed deterioration.

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[2] Numbers in parentheses refer to list of references.

Applications of the GADA Test

This test appears to be most applicable to seed lots of corn, sorghum, small grains, and grasses. Its use should be restricted to these seeds until more information is obtained on other types of seeds.

Monitoring deterioration in storage

As seeds age in storage, a decrease in GADA can be detected before germination is affected. This characteristic can be used as the basis for monitoring the condition of seed lots in storage by the following method suggested for hybrid seed corn:

The first step is to establish normal GADA deterioration curves for each inbred or hybrid and each storage facility. Do this by measuring the GADA when the seed is put in storage and each spring and fall thereafter. Conduct cold tests and field performance trials concurrently with the GADA testing to establish correlations between GADA and the first drop in field performance (stands and yields).

Next, begin a systematic GADA testing program for all new lots, testing at the beginning of storage and at approximate intervals thereafter.

Then, compare the deterioration rate of lots currently in storage against the normal rate. Lots that have reached the allowable limit of deterioration should be disposed of. Lots with harmless levels of deterioration may be continued in storage.

The use of a control chart for monitoring GADA is illustrated in Figure 1. Here it is assumed that the seed has been placed in storage in October. The solid line (standard) represents normal GADA as determined by past experience (each line or hybrid may be different). The dotted line for Lot B indicates that GADA for the lot is considerably below standard. This may indicate that the storage conditions are below standard or that the storability of Lot B is poorer than normal (possibly because of poor field conditions). Corrective action can be taken to prevent the rapid deterioration of future lots as occurred in Lot B. The dotted line for Lot A indicates that this lot is storing better than normal and possibly could be carried over in storage for another year. The degree of deterioration that lowers the performance potential of the seed must be determined by experience.

Predicting seed storability

It has been observed that certain seed lots decline in viability and vigor at a faster rate than others of the same kind stored under similar conditions. Differences in longevity of seed lots under the same storage environment are related to the condition of the seed when it was put into storage. GADA can be used as a sensitive indicator of the original condition of the seed and hence of the relative storability of the seed. In general, the lower the GADA, the shorter the potential storage life of the seed.

Again, it will be necessary to compile information under commercial conditions on which to base storability predictions. To do this, determine the GADA on seed lots as they are placed in storage. Then compare germination and cold test records after 1, 2, or more years with the original GADA levels. With experience, it should be possible to determine the approximate level of GADA which indicates poor storability.

Factors Affecting GADA in Seeds

Varieties and hybrids differ in the level of GADA present in fresh high quality seed. These varietal differences in GADA do not appear to be related to performance. Therefore, standard levels of GADA must be determined for each variety and comparisons must be made between lots within the same variety and not between varieties.

All the factors that affect GADA are not yet known. However, it is possible that soil conditions and field environment may affect the level of GADA. These factors and their affect on seed quality and longevity should become apparent as testing programs progress.

Procedure for Testing

In precise scientific research, GADA is usually determined by expensive and time-consuming colorimetric, electrophoretic, or manometric techniques (1, 2, 3, 6, 8). It is felt that these methods are not practical for use in most seed quality control programs. The method described here is rapid and inexpensive, and is adapted from a method first proposed by Linko (7) for determining GADA in high moisture wheat grain in storage.

Equipment Needed

The items of equipment needed to perform the test are as follows:

1. Grinder
2. Water bath
3. Time clock
4. Torsion balance
5. Stirring rod
6. Liquid dispenser
7. Pinch clamps
8. Respirometers

Grinder

The seed must be finely ground. This can be done with a Wiley Mill with a 20 mesh screen, a Waring blendor, or any other type of grinder that will uniformly grind seed to a small particle size.

Water bath

It is extremely important that tests be run at a uniform temperature since the results will vary with a small difference in temperature. A bath measuring approximately 13 in. x 12 in. x 7 in. deep is a convenient size.

Time Clock

A time clock with a sweep second hand is best for accurate timing.

Torsion balance

The balance should be capable of accurately weighing to 2 places to the right of the decimal point.

Stirring rod

Should be of glass or plastic.

Liquid Dispenser

The liquid should be measured to the nearest 0.1 milliliter. It is recommended that this be done with a 50 ml. burette graduated in intervals of 0.1 ml.

Pinch clamps

Simple clamps to pinch off the rubber tube on the air vent. One needed for each respirometer.

Respirometers

The respirometers are not available commercially but may be easily constructed. Materials needed for one respirometer are: a small-mouth half-pint mason jar, No. 12 rubber stopper, a 48-inch capillary tube, a 300 mm. plastic scale (a foot ruler, calibrated both in inches and millimeters), 2-1/2-inch length of 6 mm. outside diameter glass tubing, 3-inch section of 3/16-inch inside diameter rubber tubing, a pinch clamp.

The manometer is constructed by bending the capillary tubing as illustrated in Figure 2. The bends are easily made by heating the area to be bent over a bunsen burner or similar heat source until the glass is flexible. Make the arm with the ruler about 21 inches long and the arm inserted in the rubber stopper about 15 inches long; however, these measurements may vary somewhat without affecting the performance of the manometer.

Drill two holes in the rubber stopper with a No. 3 cork borer. Locate the holes as shown in the illustration. Insert the manometer tube in one hole and the short length of glass tubing in the other. Apply a little water or vaseline to the glass for easier insertion. Slip the rubber tubing over the short glass tube. This serves as an air vent. Glue or scotch tape the ruler to the upper arm of the manometer tube.

Fill the manometer tube with Brodie's solution to the level shown in Figure 1. Do this by immersing the upper end of the tube in the solution and sucking in with the mouth until the liquid is drawn up the entire length of the upper arm, to an inch around the bend. When the manometer is returned to an upright position, the fluid will assume the position shown in Figure 2. Do not let air bubbles enter the tube or it will not read correctly.

It is best to construct about 12 respirometers so several tests can be run at a time.

Solutions

Glutamic Acid

The glutamic acid solution is made by mixing glutamic acid with a buffer solution. Buffer solution is used instead of water to keep the pH at the proper level.

First mix the buffer solution as follows: Mix stock solution A by dissolving 9.08 grams dry KH_2PO_4 (monobasic potassium phosphate) in 1000 milliliters water. Mix stock solution B by dissolving 9.47 grams dry Na_2HPO_4 (dibasic sodium phosphate) in 1000 milliliters water. Prepare solution C by mixing 16.5 milliliters solution A with 183.5 milliliters solution B. Solution C will have a pH of approximately 5.8 which is proper for this test.

Prepare the glutamic acid solution by mixing 1.471 grams glutamic acid in 100 milliliters of solution C.

The buffer solutions may be stored for long periods of time, but the glutamic acid solution should be prepared fresh each day.

Brodie's Solution

Brodie's solution is the indicator liquid in the manometer tube. To make it, dissolve 23 grams of sodium chloride (table salt), 5 grams of sodium choleate, and 100 milligrams of Evans blue in water, and dilute to 500 milliliters.

Procedure for Performing the Test

1. Grind about 35 grams of air-dry seeds in the Waring blendor until the seeds are finely pulverized. This will take one to two minutes, the exact time depending on the kind of seed. If a Wiley Mill is used for grinding, use a 20-mesh screen.

2. Weigh 30 grams ground seed and place in respirometer jar.

3. Add 15 milliliters glutamic acid solution.

4. Mix ground seed and glutamic acid solution immediately with a glass rod. Mix rapidly until all ground material is wet.

5. Place manometer on jar, press rubber stopper firmly to seal jar to prevent leakage.

6. Place respirometer in 30° C water bath and record the time.

7. Allow a 10-minute equilibration period for respirometers and seed to attain the same temperature as the water bath.

8. Close air vent with pinch clamp.

9. Record height of Brodie's solution in millimeters.

10. Allow test to run 30 minutes. In some samples, the Brodie's solution may reach the top of the manometer before 30 minutes. In this case, terminate tests after 25 minutes or even 20 minutes, if necessary.

11. Record height of Brodie's solution after 30 minutes. The difference in height is the amount of carbon dioxide produced by 30 grams of seed in 30 minutes at 30° C.

12. Place a respirometer without seed in the water bath at the beginning of each test to serve as a thermobarometer to correct for changes in temperature and atmospheric pressure during a test. If the thermobarometer declines 10 millimeters during a 30-minute test, 10 millimeters should be added to the reading obtained. If the thermobarometer rises 10 millimeters during the test, 10 millimeters should be subtracted from the reading

13. Conduct all tests in duplicate, and average the results of the 2 tests.

14. A form for recording all readings and calculations is illustrated in Figure 3. The method of recording data will become evident after studying the two examples given.

List of Equipment and Chemicals Needed

Item	Specifications	Approx. cost	Coded Source
Waring blendor	single speed	35.00	M, L, F
Water bath	general purpose	135.00	M, L, F
Time clock	Kodak	10.00	M, L, F
Torsion balance	with weight dials	210.00	M, L, F
Stirring rod	glass, 8 inch	.05	M, L, F
Buret	analytical, with stopcock	6.00	M, L, F
Pinch clamp	Day pinchcock	.20	M, L, F
L Glutamic acid		1.90/200 grams	N
Potassium phosphate	monobasic, certified or reagent grade	2.35/lb.	F
Sodium phosphate	dibasic, anhydrous certified or reagent grade	3.25/lb.	F
Sodium chloride	granular	.95/lb.	F
Sodium choleate		3.90/100 grams	N
Evans blue		2.10/10 grams	F
Capillary tubing	1 mm. (3/4-1 1/4) bore	12.85/5 lb.	M, L, F
Glass tubing	6 mm. outside diam.	1.00/lb.	M, L, F
Rubber tubing	3/16 in. inside diam. 3/32 in. walls	1.75/8 ft.	M, L, F
Ruler	12. in, plastic or wood, millimeter scale	.10	M, L, F

Names and Addresses of Sources

There are many supply houses that can provide the equipment and supplies needed. It is not practical to list them all and the following are only suggestions.

Coded Source	Names and Addresses
M	Matheson Scientific, Inc. 1735 North Ashland Avenue Chicago, Illinois 60622 Phone (312) 278-4630
L	LaPine Scientific Company 6001 Knox Avenue Chicago, Illinois 60629 Phone (312) 735-4700
F	Fisher Scientific Company 1458 N. Lamon Avenue Chicago, Illinois 60651 Phone (312) 261-1221
N	Nutritional Biochemicals Corp. 26201 Miles Road Cleveland, Ohio 44128 Phone (216) 662-0212

References on GADA

1. Bautista, G. M. and Pekka Linko. 1962. Glutamic acid decarboxylase activity as a measure of damage in artificially dried and stored corn. Cereal Chem. 39:455-458.

2. Bautista, G. M., J. C. Lugay, Lourdes J. Cruz, and B. O. Juliano. 1964. Glutamic acid decarboxylase activity as a viability index of artificially dried and stored rice. Cereal Chem. 41: 188-191.

3. Cheng, Yu Yen. 1959. The kinetics and occurrence of wheat glutamic acid decarboxylase. M. S. Thesis, Kansas State Univ.

4. Grabe, D. F. 1964. Glutamic acid decarboxylase activity as a measure of seedling vigor. Proc. Assoc. Off. Seed Anal. 54: 100-109.

5. Grabe, D. F. 1965. Prediction of relative storability of corn seed lots. Proc. Assoc. Off. Seed Anal. 55:92-96.

6. Linko, Pekka. 1960. Water content and metabolism of wheat during short storage and germination. Ann. Acad. Sci. Fennicae A. II. Chemica 98. 69 pp.

7. Linko, Pekka. 1961. Simple and rapid manometric method for determining glutamic acid decarboxylase activity as quality index of wheat. Jour. Agr. & Food Chem. 9:310-313.

8. Linko, Pekka and Lars Soqn. 1960. Relation of viability and storage deterioration to glutamic acid decarboxylase in wheat. Cereal Chem. 37:489-499.

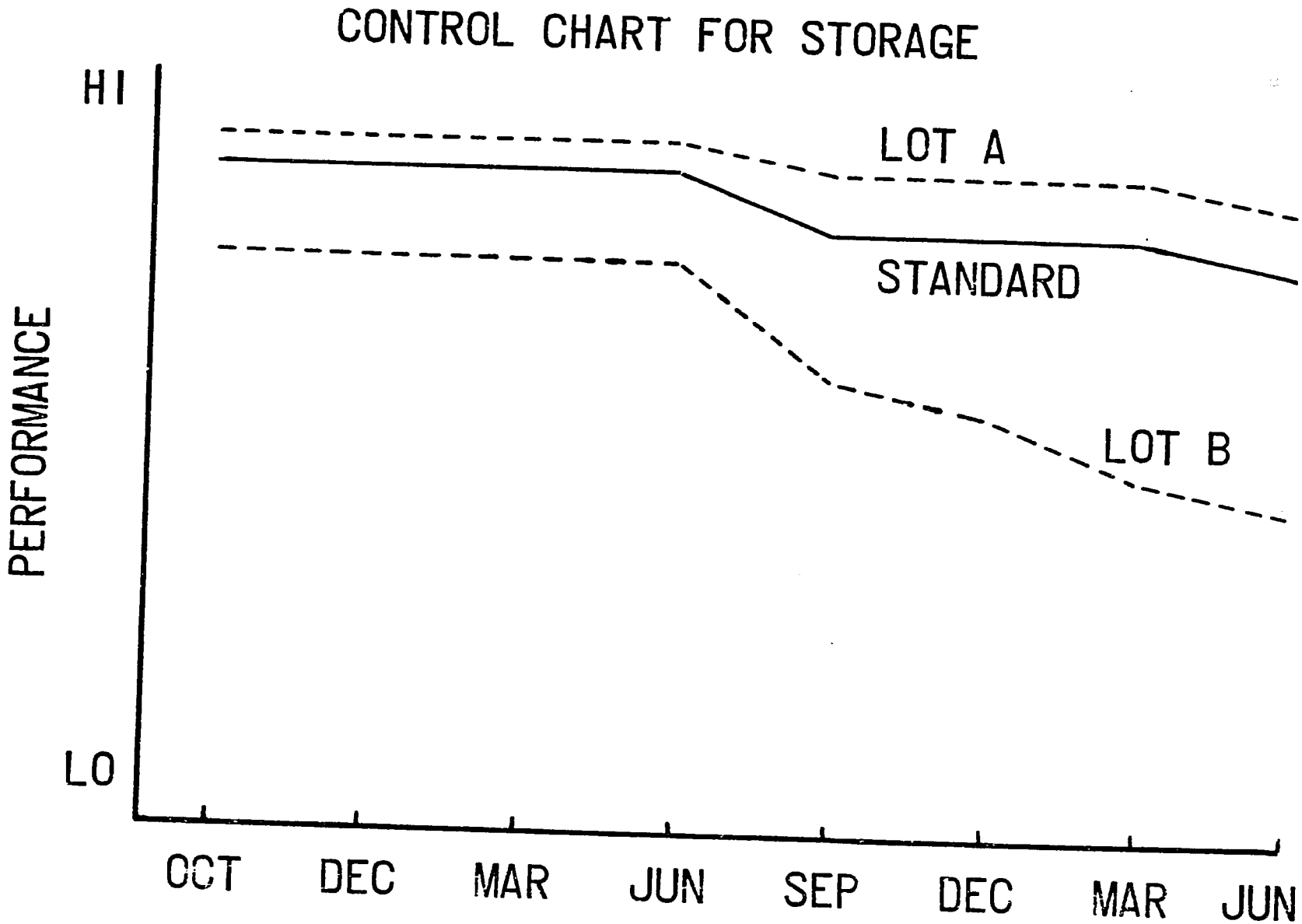

Figure 1. Example of control chart for monitoring GADA of corn seeds in storage.

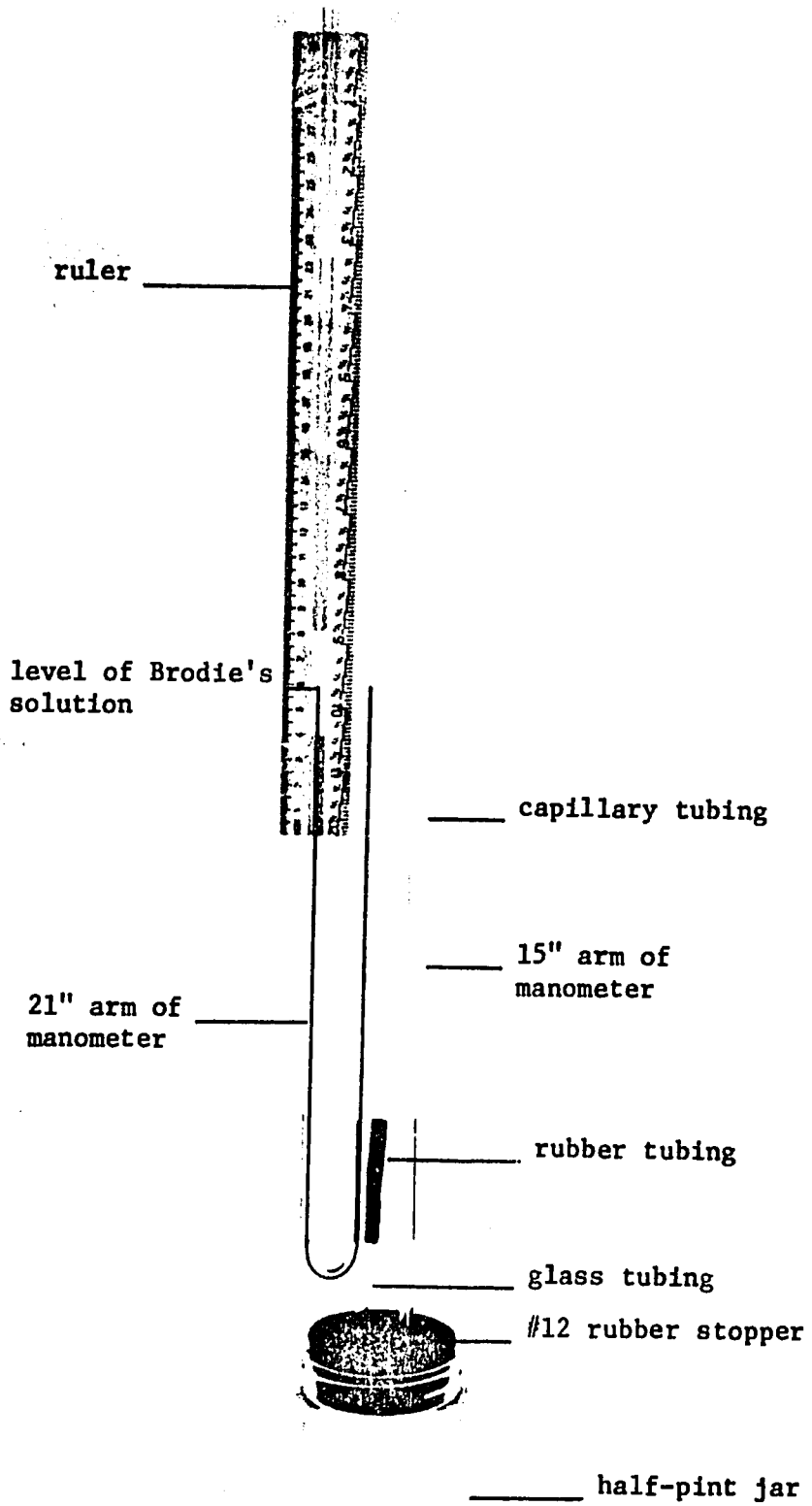

Figure 2. Respirometer utilized in determinations of GADA.

Figure 3. SAMPLE DATA SHEET FOR GADA TEST

Manometer Number	Sample Identification	Time				Manometer Reading			Thermobarometer			Corrected Reading	Average
		vent open		vent closed									
		start	end	start	end	original	final	change	original	final	change		
1	Lot A	0	10	10	40	54	204	150	74	78	+4	146	
2	Lot A	[illegible]	13	13	43	49	194	145	71	75	+4	141	144
3	Lot B	42	52	[illegible]	[illegible]	[illegible]	171	[illegible]	[illegible]	[illegible]	−5	125	
4	Lot B	45	55	55	85	42	182	140	72	68	−4	130	128

(2)