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The GADA Test for Seed Storability

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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.

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.

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2/ Numbers in parentheses refer to list of references.

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 the 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 1. 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;

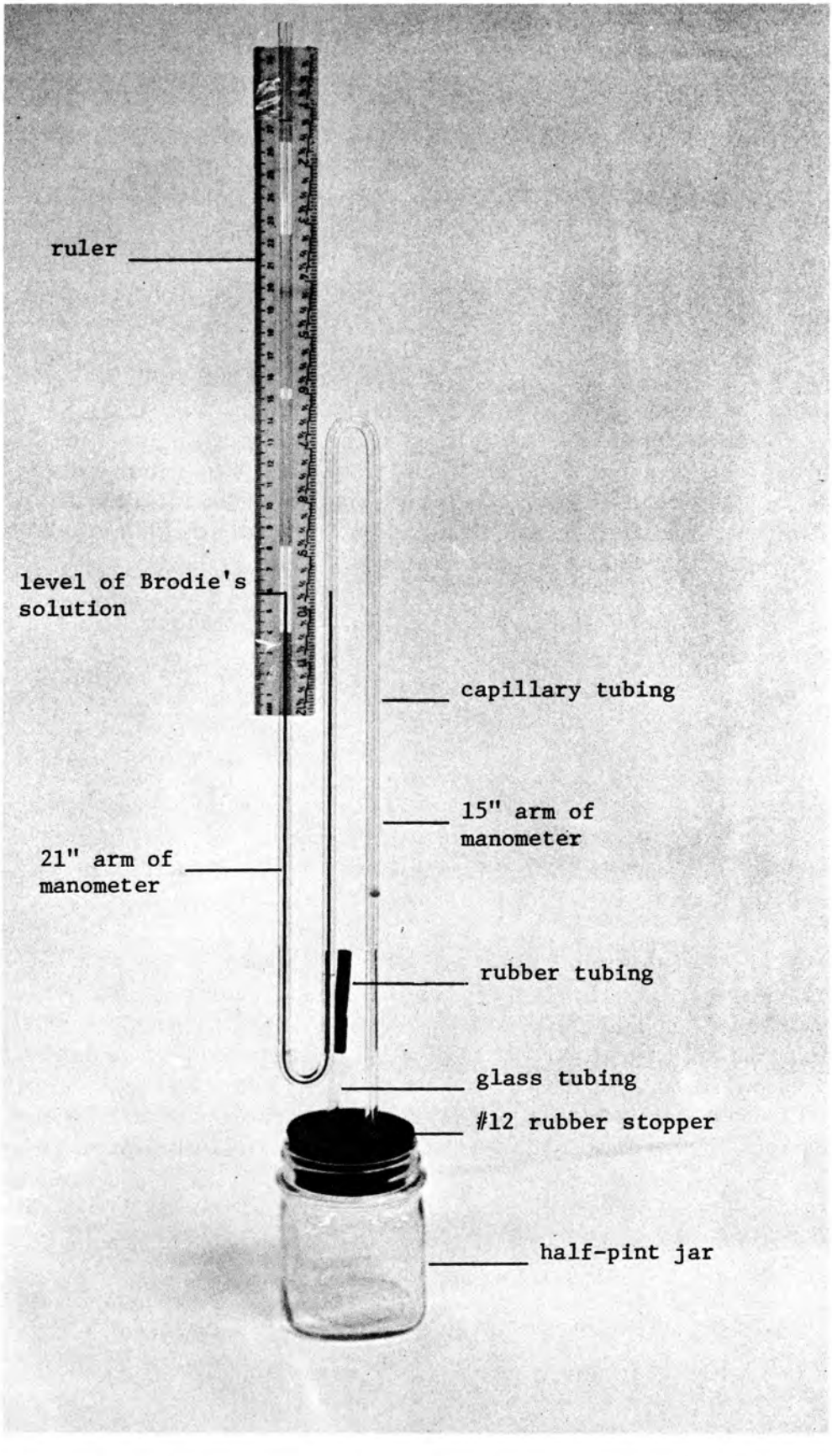

Figure 1. Respirometer utilized in determinations of GADA.

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. 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 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 munutes 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.

List of Equipment and Chemicals Needed

Item	Specifications	Coded Source
Waring blendor	single speed	M,L,F
Water bath	general purpose	M,L,F
Time clock	Kodak	M,L,F
Torsion balance	with weight dials	M,L,F
Stirring rod	glass, 8 inch	M,L,F
Buret	analytical, with stopcock	M,L,F
Pinch clamp	Day pinchcock	M,L,F
L Glutamic acid		N
Potassium phosphate	monobasic, certified or reagent grade	F
Sodium phosphate	dibasic, anhydrous certified or reagent grade	F
Sodium chloride	granular	F
Sodium choleate		N
Evans blue		F
Capillary tubing	1 mm. (3/4-1 1/4) bore	M,L,F
Glass tubing	6 mm. outside diam.	M,L,F
Rubber tubing	3/16 in. inside diam. 3/32 in. walls	M,L,F
Ruler	12 in. plastic or wood millimeter scale	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 then 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

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