Cottonseed Quality Evaluation

Introduction

There are several tests for evaluating cottonseed quality. Some tests use speed for a quick estimate, while others may be more accurate but require more time and labor. Effective evaluation requires a number of different tests. Several tests of the same kind at different times may be necessary for satisfactory evaluation.

Cottonseed evaluation should begin as seed are received at the storage area. Immediately determine if seed are to be for planting, or reject them, in which case they would go to some use other than planting seed. Those accepted for planting seed are stored and generally tested several more times before delinting, conditioning, treating, and bagging.

Tests that are applicable for rapid evaluation (during a period of about 30 minutes) include cutting, visible mechanical damage, free fat acidity (when a press is used to extract oil), seed coat maturity (visual), measuring temperature of the seed (in bulk on the carrier), and measuring seed moisture content.

Later, conduct standard germination, tetrazolium, and cool germination tests. Germination and tetrazolium tests are usually conducted during the storage period, before the delinting process begins. After conditioning, treating, and bagging, final germination and cool germination tests determine if the seeds are suitable for sale.
Tests and Procedures

Cutting Test

The cutting test is for a quick estimate of germination of gin-run cottonseed. With experience, you can get a fairly good estimate of germination. You need a cutting bar, or some other means of holding the seeds in place, and a cutting instrument for the cutting test. Randomly select a minimum of 100 seeds from the sample, place in the bar, then cut and evaluate the cut seeds.

Good seeds have a whitish to yellowish-green color (figures 1 and 2). Immature seeds are easily recognized, because they are empty or have small, sometimes shriveled embryos (figure 3). The dark seeds are not germinable (figures 4 and 5). When seeds appear brown to brownish yellow, the quality is poor (figure 6). These seeds may not germinate or may appear weak and develop late in the germination test. The difficulty in evaluation is with seeds that are marginal in color, as those in figure 6.

Visual Mechanical Damage Test

Visual mechanical damage should also be used to evaluate seed quality, especially where seeds are to be acid-delinted. Cut or cracked seed coats allow the entry of acid, which can severely damage the embryos and may even kill the seeds. Hand acid-delinting a sample of gin-run seeds and randomly selecting 200 to 400 seeds for evaluation generally are adequate. Some type of magnification and a good light are necessary for evaluation.

Damaged seeds may be classified as to the severity of damage (ARS, 1972). This is important, because the more severe the damage, the greater the chance the seeds are of no value. The following classifications are suggested:

No damage - seeds with completely intact seed coats.

Pinhole damage - seeds with only one or two small (pinhole) punctures in seed coats.

Minor damage - seeds with seed coats cracked or cut, but not severely; damage primarily to the chalazal end or on sides of the seed.

Major damage - seeds with large cuts or ruptures in the seed coats, part of the seed coat missing, cotyledons exposed, or damage to the radicle end of the seeds.

The four categories are shown in figures 7, 8, 9, and 10. Undamaged and pinhole-damaged seeds are not adversely affected by acid-delinting when delinted properly. Minor-damaged seeds may germinate but are generally of low quality. Major-damaged seeds usually do not survive the delinting process.

Seed Coat Maturity

Seed coat maturity is also an important characteristic in determining seed quality. Immature seeds are not as high in quality as are the matured seeds. Seed coat color of mature seeds ranges from dark brown to black, depending on the variety. Thickness of seed coat also varies with variety. You must be familiar with the color of the mature seeds of the variety in question; even then, you may have difficulty detecting slightly immature seeds. However, the real problem is the grossly immature seeds with the white or very light seed coats. These can be visually separated in a hand acid-delinted sample. The number of immature seeds in the sample selected for mechanical damage evaluation generally will be satisfactory for an estimate of the percentage of seeds with immature seed coats.

Maturity is also detected in the cutting test previously described. You should get a good estimate of maturity of the sample in question with the cutting test immature count, combined with seed coat evaluation. Immature seed may germinate, but they are lower in quality than are the mature seeds.

Standard Germination

The standard germination test is basic in an evaluation program. Everything else revolves around germination. If germination is not up to the standards set, then nothing else matters. Germination may be acceptable, however, and the seeds still not be suitable for conditioning or for marketing. Conduct the standard germination tests according to the Association of Official Seed Analyst (AOSA), Rules for Testing Seed, or the International Seed Testing Association (ISTA), Rules for Testing Seed.

Substrata are usually rolled towels, but blotters, sand, or soil also may be used. Take care not to have substrata too wet or allow them to become too dry during the test period.

Temperature for germination, specified by AOSA and ISTA rules for Testing Seed, is an alternating

Using AOSA rules, seedling counts are specified at 4 and 12 days at 20 - 30°C, and 4 and 8 days at 30°C. However, these times are approximate, and the first count may deviate one or more days. The test may be terminated before the number of days specified, if the analyst is positive the maximum germination of the sample has been attained. The test period may also be extended 2 days, if necessary, to allow sufficient seedling development for positive evaluation (AOSA, 1991).

At the first count, remove only normal seedlings. The primary purpose of the first count is to reduce the amount of material on the germination medium. Normal seedlings, to be removed at the first count, are shown in figures 11 and 12. All have well-developed hypocotyl and radicles. In sand or soil, you need to make only one count.

At the final count, all remaining seedlings (or seeds) are evaluated and classified. Seedlings considered as normal at the final count are shown in figures 13 and 14. Seedlings in figure 14 do not have a primary root, but have a secondary root system well enough developed to sustain the young plant. Seedlings considered abnormal at the final count are shown in figures 15 and 16. These seedlings do not have an adequate secondary root system or have no root system at all, or the root hypocotyl is diseased.

Drawings and descriptions of normal and abnormal seedlings of the family Malvaceae may be found in the Seedling Evaluation Handbook of the Association of Official Seed Analyst (AOSA, 1992). Although not indicated in the rules, hard seeds may occur in cotton. The same rule applying to hard seeds in other species applies to cotton, i.e., hard seeds are added to the final count of normal seedlings to calculate percentage of germination (PMA, 1952). Physiological dormancy may also occur, especially in gin-run seeds shortly after harvest and in seeds after delinting in the early part of the delinting season. This type of dormancy produces a “firm seed,” one that has imbibed water but does not germinate. Physiological dormancy can be overcome by placing dry seeds at 50°C for 24 hours, before planting the germination test.

### Tetrazolium Test

The **tetrazolium test** is an enzyme reaction in which live tissues stain red, and dead tissues do not stain (Delouche et al., 1951). It can be used to estimate seed germination and vigor and can be a useful tool in determining the nature and extent of seed quality problems during harvesting, conditioning, storage, and distribution.

The basis for viability and vigor evaluation involves the location, identification, and appraisal of sound and weak or dead embryo tissues, because these tissues relate to seedling development and overall strength of the developing seedling.

The analyst must have a knowledge of seedling structure, i.e., what constitutes normal and abnormal seedlings and what parts of the embryo develop into the respective seedling structures (figure 17).

In the preparation of seeds for staining, the seed coat and the membrane surrounding the embryo must be removed before placing them in the tetrazolium solution. It is necessary to condition seeds by softening the seed coats before attempting to remove them.

Gin-run (fuzzy) seeds may be soaked in free water for 12 to 18 hours (overnight). Place the sample to be tested in a container, cover with water, and let the sample stand at room temperature until ready for seed coat removal. Seeds that have been delinted, particularly acid-delinted, are best conditioned by rolling them in wet germination towels for 12 to 18 hours (overnight). Soaking acid-delinted seeds in free water may result in an erroneously low estimation of germination if there is an acid residue on the seed coats. When seeds are rolled in towels for preconditioning, acid residue does not present a problem.

The removal of the seed coat and membrane is best accomplished using sharp-pointed tweezers, beginning at the chalazal end. When the seed coat is removed, drop the embryo into a container of water; this helps loosen the membrane surrounding the embryo. When seed coat removal is completed, remove the membrane, taking care not to break the radicle, or otherwise damage the seed with the tweezers. Place the seeds back in water.

When membrane removal is completed, drain water from seeds, cover with tetrazolium solution, and place in an oven at 40°C for approximately 1 hour. The strength of the tetrazolium solution used is the...
Figure 1. Germinable seed, *Gossypium hirsutum* L.

Figure 2. Germinable seed, *Gossypium barbadense* L.

Figure 3. Immature seed

Figure 4. Nongerminable seed, *Gossypium hirsutum* L.

Figure 5. Nongerminable seed, *Gossypium barbadense* L.

Figure 6. Marginal seed, *Gossypium hirsutum* L.

Figure 7. Undamaged seed

Figure 8. Pinhole mechanical-damaged seed

Figure 9. Minor mechanical-damaged seed

Figure 10. Major mechanical-damaged seed

Figure 11. Normal seedlings, first count

Figure 12. Normal seedlings, first count
Cotton Seed and Seedling

Seed

chalazal end
radicle end

Seedling

cotyledons
ttrue leaf
hypocotyl

Embryo

cotyledons
radicle

Cross Section

membrane
radicle
cotyledons

Germinating Seedling

cotyledons
hypocotyl
seed coat
radicle

primary root
(tap root)
secondary root

Figure 17. Cottonseed and seedling; identification of related parts

choice of the analyst. Most analysts use a 0.5- to 1.0-percent solution; however, other concentrations may be used. Some analysts prefer weaker solutions. The 1-hour staining time is based on using a 0.5-percent solution. Stronger solutions require less time, weaker solutions more time. After you have reached the desired level of staining, drain the tetrazolium solution and rinse seed two to three times in cold tap water. Cover seeds with water until evaluation. If evaluation is not made immediately, place in a refrigerator. The seeds can be held up to 24 hours under refrigeration, if necessary, before evaluation. Evaluate under magnification and good light. Some analysts prefer higher magnification, such as that provided by a stereoscopic microscope. In the evaluation process, the embryos must be thoroughly examined, especially the radicle. Part the cotyledons with tweezers in order to see the entire radicle. Cutting the embryo with a single-edged razor blade can help in the evaluation; this allows you to “see” inside the embryo, if necessary.

The most desirable color for cottonseeds is a dark pink to a light red. Darker seeds may also be
germinable. Germinable seeds may be completely stained, or the seeds may have slight, small dead areas over the cotyledon or the chalazal end, or have dead or weak tissue over less than one-third of the cotyledonary area. The radicle tip only can also be dead. The radicle tip in a good seed may be quite dark, because this is an area of high-metabolic activity, and the small amount of tissue allows deeper penetration of the tetrazolium solution, resulting in a dark appearance. Do not mistake this for weak tissue.

Nongerminable seeds include:
• Those with one-third or more of the radicle unstained;
• Those with more than one-half of the cotyledonary tissue unstained;
• Those stained dark red to purplish red;
• Those stained grayish red or are milky in appearance; and
• Those completely unstained.

The dark or milky seeds may also be somewhat soft and flaccid. Immature seeds may stain a desirable color, but may be somewhat soft and flaccid, and the cotyledons may be beginning to unfold or be nearly unfolded. If these seeds germinate, they will be weak and of little value. Figures 18, 19, 20, and 21 show germinable and nongerminable seeds as they are placed in vigor categories. Vigor of cottonseeds may be determined as discussed in this publication (Metzer, 1961; Lago, 1975).

High-vigor seeds are completely stained or have only minor unstained areas on or near the chalazal end of the seed. Staining is uniform and bright, not deeply stained, tissue is firm, and the radicle tip is stained, but is darker than the cotyledons (figure 18).

Medium-vigor seeds may have minor unstained or darkly stained areas over various portions of the cotyledons; the radicle-hypocotyl is uniformly stained, but not dark, except for the radicle tip. The extreme tip of the radicle may be unstained. Tissue is firm, but may be slightly darker than high-vigor seeds (figure 19).

Low-vigor seeds may have large but not essential areas of the cotyledon (area next to the radicle) unstained. The tip of the radicle may be unstinted into the extreme tip of the vascular tissue (stele). Cotyledons may be darkly stained or with a slight milky appearance in parts of the cotyledons. Tissues may be somewhat flaccid (figure 20).

Nongerminable seeds include those with one-third or more of the radicle extremely dark (almost black) or unstained, one-half or more of the cotyledonary tissue unstained, entire seeds stained dark, seeds stained grayish or cloudy (milky), seeds with one-third or more of the radicle missing, and very flaccid embryos (figure 21).

To classify seeds as strong or weak, combine the high- and medium-vigor categories.

The tetrazolium test does not detect the presence of disease organisms. It does not detect overtreatment with fungicides or other materials that do not cause damage until germination begins. The tetrazolium test is also useful in “trouble shooting.” Figures 22, 23, and 24 show mechanical damage, acid damage, and heat damage, respectively.

Conductivity Test

The conductivity test measures the electrical conductivity of the water in which seeds have been soaked. As seeds deteriorate, cell membranes begin to break down, allowing cellular content to “leak.” The electrical conductivity of the steep water, containing leachate from the deteriorated seeds, produces a different reading than that from high-quality seeds where there is little leachate in the steep water.

Mechanical damage to a seed also results in more material being leached into the steep water; thus, a seed with a damaged seed coat gives a reading similar to that of a deteriorated seed without mechanical damage.

Seed treatment materials, fungicides and insecticides, may also affect the amount of materials in steep water, thus affecting conductivity readings.

The equipment for measuring conductivity is highly specialized and sophisticated. Machines capable of measuring conductivity of as many as 100 cells at a time, with digital readout and printout capabilities, are available. Specific instructions for seed preparation, operation, and interpretation for specific models come with the instruments.

Hopper (1986) indicates that conductivity was related to germination, emergence potential, and early seedling growth.

Free Fat Acidity

Free fat acidity is used quite extensively as an index of seed quality. This test is based on the break-
down of fats and oils to fatty acids and glycerol as seed deterioration progresses. Free fatty acids usually build up under high temperatures and high-seed-moisture conditions. Once seeds are dry—10 percent or less moisture—free fatty acids will not increase. The level might actually decrease slightly: 0.1 percent or so when stored under cool, dry conditions. Deterioration is not always accompanied by an increase in free fatty acids, however, and seeds above 1.0 percent in free fat acidity may germinate. A seed lot may germinate poorly or not at all and be well below 1.0 percent in free fat acidity. Free fat acidity is best used to indicate variation among seed lots, or to detect variation in seed quality and possible problem lots. The 1.0-percent level of free fatty acids is the most common acceptable upper level for seeds. However, as has already been mentioned, seeds well below 1.0 percent may be of poor quality. On the other hand, seeds at 1.5 percent or higher free fatty acid content may be acceptable for planting. There may be times when most of the seeds available are above 1.0 percent free fatty acid, and there is no choice but to save these seeds.

An example of when to use free fat acidity is: if seeds are being checked as they are transported to a warehouse for storage; and free fat acidity is regularly in the range of 0.5 to 0.8 percent, and suddenly samples begin to run 1.2 or higher percent, these seeds should be rejected, or at least stored in a separate area until they can be further evaluated. Certainly seeds that are high in free fatty acids should not be carried over to the next planting season.

The solvent method of extracting oil is not practical for most seed companies. However, there are hydraulic presses that will extract small quantities of oil. These presses are suitable for most seed companies. In addition to an oil press, you need other equipment and supplies.

**Low-Temperature Germination Test**

Low-temperature germination, referred to as the cool-germination test, can also be used as an indication of physiological quality (AOSA, 1983). As indicated by its name, this test subjects seeds to cool, wet conditions and is intended to represent cool, wet field conditions. One major difference, however, is the absence of the soilborne organisms that cause seed rot and seedling diseases. The test does measure differences in seed lots and provides valuable information.

This test requires the maintenance of a constant 18 °C (64.4 °F) ± 0.5 °C. Maintaining constant temperature is essential. If the temperature is allowed to fluctuate up to 20 °C (68 °F), much of the effects of the test will be lost. If it fluctuates to 16 °C (60.8 °F), seedling growth is significantly reduced. **If this constant temperature cannot be maintained, the test is of no value.**

Preparation of seeds for testing is the same as for a standard germination test (seeds are rolled in moist germination towels). Generally, 200 seeds from each lot are tested in four replications of 50 seeds each. Rolled towels are placed on end (upright) in a wire or plastic mesh container, or plastic crisper with a cover. If a dry cabinet is used, cover containers to prevent towels from drying during the test.

Only one count is made on the seventh day. All normal seedlings having a combined root-hypocotyl length of 1 1/2 inches (3.75 cm) or longer are counted. The root-hypocotyl measurement is made from the tip of the radicle (primary root) to the point of attachment to the cotyledons. These are considered the vigorous or strong seedlings. Use this test only on seeds that have been delinted and treated with a fungicide. Do not use it for gin-run seeds.
Literature Cited


do Lago, A. A. 1975. Development of equations to predict the storability of gin-run cotton [Gossypium hirsutum (L.)] seed lots. Thesis (M.S.), Mississippi State University, Mississippi State, Mississippi.


Figures

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Publication 1978
Extension Service of Mississippi State University, cooperating with U.S. Department of Agriculture.
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