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Quality Assurance Techniques

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QUALITY ASSURANCE TECHNIQUES

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Emphasis: Vigor Tests

ACCELERATED AGING TEST

The accelerated aging (AA) test is useful in determining the storage potential of seed lots. High germination of a particular seed lot is not assurance that the lot will keep well in storage. Seed lots of equal germination do not have equal storage potential. Seed lots deteriorate rapidly under high temperatures and humidity. High temperature (approximately 42C) and a relative humidity close to 100% is used on a sample of any particular seed lot to obtain a pre-view of what the germination of that lot may be if it is put into storage for six months, one year or longer.

Material needed:

1. The main item needed is an accelerated aging chamber in which the temperature can be maintained at approximately 42C and a relative humidity close to 100%. Some arrangement must be provided to prevent condensate on the inside of the chamber from dripping on seed stored in the chamber. To maintain a high humidity it is necessary to keep a supply of water in the bottom of the chamber. The heating unit should either be installed below or in the reservoir of water so moisture "driven" off the surface of the water during heating will maintain the humidity of the chamber.

2. Containers which will hold 100 seeds of the kind(s) to be tested must be provided. These containers can be made of galvanized mesh wire or other material which will not inhibit air flow to the seeds.

Procedure:

1. Ordinarily four replicates of 100 seeds each of the seed lot(s) to be tested are counted and transferred to the AA chamber. Seventy two hours has been used for the conditioning period in the AA chamber with good success. However, any one intending to use the AA test should plan to conduct experiments to learn the length of time required to obtain meaningful results.

2. After the conditioning period the four 100 seed replicates are planted for germination in the usual way.

3. At the end of the regular test period germinated seedlings are counted. Generally a somewhat less critical application of germination standards are applied. Any seed is counted as germinable if it produces an identifiable root and shoot regardless of size or condition.

4. If desired, at the time the conditioned seeds are planted for germination 4 x 100 non-conditioned seeds from the same lot may be planted for a regular germination. It is sometimes helpful to compare the seedlings and the germination of conditioned and non-conditioned seed.

Conclusions:

The AA test can be used to predict the storage potential of seed lots. This test can be especially helpful to a seedsman who has several lots of the same variety and all of the seed of that variety cannot be sold before the planting season. The results of the AA tests on all his lots should provide the answer.

Acknowledgement:

The essential information for this write-up was taken from the paper by Delouche, J.C. and C.C. Baskin 1973. Accelerated aging techniques for predicting the relative storability of seed lots. Seed Science and Technology 1 (2): 427-451.

THE COLD TEST FOR CORN

The objective of the cold test is to evaluate the potential field emergence of corn seed under unfavorable field conditions (cold and wet).

Materials needed:

1. Plastic crisper boxes 7 1/2" x 10 1/2" x 4" with flanged lids have been used. However, any comparable sized (or somewhat larger) container with a tight fitting lid may be used.

2. A non-sterile sand-soil mixture consisting of two parts sand and one part soil are used. Care is used to obtain soil that has not been treated with an herbicide. The soil is screened through a 1/4" mesh screen to remove debri, then placed in a metal lined bin at room temperature and allowed to stabilize for two to three weeks before use. After the soil has stabilized, air dry mason sand is mixed with it in proportion of 2 parts sand and 1 part soil.

Procedure:

1. One measure (approximately one pint) of the media is placed in the container and evenly spread in the bottom. One hundred seeds are distributed on this layer, pressed down to keep the seeds in place and insure contact with the media. One measure of the media is placed over the seed covering it to a depth of approximately 3/8". Water is added in a calibrated amount to moisten the media to 70% of saturation. No additional water is added during the test.

2. Two replicates of 100 seed are planted. If less than 200 seeds are available for testing the total amount of seed is divided into two equal parts so it is a two replicate test.

3. The containers are then covered with their lids and placed on shelves in an insulated chamber cooled to 10C. After seven days the tests are removed from the chamber and placed on growing racks at room temperature (approximately 25C) with continuous light. The test is continued for an additional five days or until the seedlings raise the lids of the boxes. Seedling counts should be made soon after the lids are raised because the media will rapidly dry. Evaluation of seedlings into normal and abnormal is based on the "Rules for Seed Testing".

Conclusion:

Hybrid seed corn seedsmen in North America base their decision to sell or discard a seed lot on data from several sources and tests, including the cold test. Usually lots in which the cold test is below 80% may be retested several times to make certain of the seed quality. Seed lots that have a high regular germination but a relatively low cold test may emerge well and have good field performance under favorable conditions.

THE SEEDLING SHOOT WEIGHT TEST

The objective of the seedling shoot weight test is to determine, under laboratory conditions, the seedling vigor of a given seed lot. This test has been used to a large extent for corn and soybeans but can also be used for other kinds of crop seed.

Materials needed:

1. Three towels, 14" x 24" are used for each replicate of the test. The towel size and weight should remain consistent for all tests.

2. Plastic waste baskets, 7" x 11" x 12" are used as containers. Partions are made by puncturing holes in the sides of the basket and drawing light copper wire through the holes and across the basket. If the holes and wires are properly placed a maximum of 12 rolled towels may be placed in each container.

3. Poly-bags 16" to 24" long and with an opening sufficiently large to fit over the basket are used to keep moisture in the containers.

Procedure:

1. Two towels are dipped in water and allowed to drip dry. The towels should be wet but a water film should not appear if the towels are pressed with the thumb. The two towels are laid on a table and two rows of 25 seeds each are placed on the towels. The top row should be approximately 1 1/2 inches below the top, the second row 3 inches below the top. The seeds are oriented with the radical end towards the bottom of the towel and the germ side of the seed down. A third dampened (drip dry) towel is placed over the seeds and the three towels loosely rolled. An elastic band is loosely fitted over the roll below the seeds. At least four replicates should be planted for each lot. Each rolled towel is placed into the container with the seeds toward the top of the container. A poly-bag is pulled down over the container. At least 12" of the bag should protrude above the container so the seedlings will have plenty of room to develop. An elastic band is slipped around the container and bag to hold the bag to the container and prevent loss of moisture.

2. The containers are ordinarily placed in a dark room at 25 + 1C for seven days. Temperatures somewhat lower or higher than 25C may be used. However, a uniform temperature from test to test should be used.

3. At the end of seven days the towels are removed and a germination count made. The seeds are "cut free" from the normal seedlings. The

normal seedlings are placed in a coin envelope and dried in an 80C oven for 24 hours. The seedlings are then weighed to the nearest mg and the total dry weight of normal seedlings per towel divided by the number of seedlings included to obtain the average seedling growth rate in mgs.

Conclusion:

When conducting seedling shoot weight tests, more than one lot must be included in the test to provide a basis of comparison. At present, the test is valid only if lots of the same variety or genotype are compared. This test has been shown to correlate well with initial seedling vigor in the field -- which may or may not relate to final yield.

COOL GERMINATION TEST OF COTTON

Basis for the Cool Germination Test:

The Cool Germination Test technique is based on the premise that when cottonseed have been subjected to a sufficiently cool temperature for a determined period of time, this will result in a decreased germination and growth rate of weak and low vigor seedlings. This test differs from the cold test which is a pathological test in that it is a cool germination test conducted at a constant temperature of 18C.

Procedure:

The test is conducted on 200 seeds from each lot. Fifty seeds are randomly placed on each of 4 double layer 11" x 13 1/2" moist towels. Two additional towels are placed over the seeds before rolling. The rolled towels are then set upright in wire mesh baskets and placed in a dark, constant 18C germinator. The seeds are placed on the towels with a vacuum counting machine in the case of acid delinted seeds and by hand for machine delinted seeds. The towels should be moist, but not so wet that by pressing, a film of water forms around the finger. Additional moisture will probably not be needed. Only one count is made on the cottonseed and this count is made on the sixth day for acid delinted seeds and the seventh day for machine delinted seeds. All seedlings having a combined hypocotyl and root 1 1/2 inches or longer and normal for this length will be considered as high vigor seedlings at the end of the test period. The measurement is made from the tip of radicle to just below seed or leaf structure. The remaining seeds or seedlings are not counted in the vigor test percentage. All seeds used in this test must have been treated with a recommended fungicide.

TETRAZOLIUM TEST

The tetrazolium test is widely recognized as an accurate means of estimating seed viability. The test is used throughout the world as a highly regarded method of estimating seed viability and is a routine test in many seed testing laboratories. It is often referred to as a "quick test," since it can be completed in only a few hours, as compared to regular germination tests that require as long as two months for some species. Tetrazolium test results can be extremely valuable for providing labeling information for immediate shipment of seed lots without waiting for completion of germination tests. It is also a valuable research technique for estimating seed viability and determining reasons for poor germination.

Principle:

The tetrazolium test distinguishes between viable and dead tissues of the embryo on the basis of their relative respiration rate in the hydrated state. Although many enzymes are active during respiration, the test utilizes the activity of dehydrogenase enzymes as an index to the respiration rate and seed viability. The highly reduced state of the dehydrogenases enables them to give off hydrogen ions to oxidized colorless tetrazolium salt solution, which is changed into red formazan as it is reduced by hydrogen ions. Seed viability is interpreted according to the topographical staining pattern of the embryo and the intensity of the coloration.

Procedure:

Seeds are first soaked in water to allow complete hydration of all tissues. For many species, the tetrazolium solution can be added to the intact seed. Other seeds must be prepared by cutting or puncturing in various ways to permit access of the tetrazolium solution to all parts of the seed . After hydration, the seeds are placed in a tetrazolium salt solution and held in a warming oven at about 35C for complete coloration. Two hours is usually adequate for seeds that are bisected through the embryo, but others require longer periods of staining. If seeds are held too long in contact with the tetrazolium solution, they tend to become overstained, making interpretation difficult. A handbook of information and instructions for performing the tetrazolium test has been published by the Association of Official Seed Analysts.

Evaluation:

Although the tissues of living seeds stain red, their interpretation as an estimate of viability requires considerable skill and experience. Sound embryo tissues absorb tetrazolium slowly and tend to develop a lighter color than embryos that are bruised, aged, frozen, or disturbed in other ways. The experienced analyst learns to distinguish between seeds with the capacity to produce normal seedlings and those that stain abnormally.

The tetrazolium test is often called the topographical tetrazolium test because of the pattern or topography, of staining is an important aspect of its interpretation. Many seeds are neither completely dead nor completely alive. The staining pattern reveals the live and dead areas of the embryo and enables the analyst to determine if seeds have the capacity to produce normal seedlings. The cell division areas of the embryo are most critical during germination, and if they are unstained, or abnormally stained, a seed's germination potential is weakened. The analyst must be familiar with crucial cell division areas of the embryo and learn to interpret their staining pattern in terms of seed germinability.

Other factors must be carefully observed when interpreting a tetrazolium test. Among these are flaccid tissues (lack of adequate turgor) and critically located fractures, bruises, and insect cavities. Any of these factors, when present in a vital position, may cause an otherwise sound seed to be nongerminable.

Source of Tetrazolium

Tetrazolium may be obtained from most chemical supply houses. One source is listed here for your information.

Fisher Scientific Company
Chemical Manufacturing Division
Fair Lawn, NJ 07410

MEASUREMENT OF LEACHATES
(Conductivity Test)

As seeds age and natural deterioration proceeds, degradation and disorganization of cellular membranes may occur, allowing nutrients to be leached from them in the presence of water. Loss of seed vigor can be detected by an increase in seed leachates in the presence of water.

Leaching in older, deteriorated seeds may be due more to available sugars (glucose, fructose, sucrose, raffinose, maltose, and xylose) than to changes in membrane structure and permeability. Better seeds with higher vigor are better able to consume soluble sugars than lower vigor seeds. This leads to a lower concentration of soluble sugars in better seeds and ultimately to a lower rate of leakage into the water medium. In contrast, the less vigorous seeds are unable to utilize these soluble sugars rapidly and lose them to the imbibing solution through diffusion, osmosis, and active transport. Leachate tests for monocotyledonous seeds, where most reserve food storage is in the endosperm, must be interpreted with great caution, since some loss of nutrients could occur without perceptible effect on vigor. In cases where slight mechanical damage occurs to the endosperm, injured seeds may leak at least twice as much sugar as uninjured seeds; yet differences in their germination may be minor. Tests with artificially aged seeds have shown that germination of aged seeds may be completely lost without any increase in their leakage of nutrients. Thus, sugar measurements are not always a reliable index of seed viability. Furthermore, the leaching of sugars may be regulated primarily by sugar utilization rather than changes in membrane permeability.

The concentration of leachates may be measured by electrical conductivity methods or by chemical methods. The conductivity test was originally developed to aid in the detection of wrinkled pea and seed lots which, although of high laboratory germination, were liable to pre-emergence failure in the field. Some lots of seed which are losing vigor release materials, such as sugars or other electrolytes, in solution into the soil which may increase the activity of soil fungi. This increase in the activity of soil fungi may interfere with the development of the seedling, especially under cold or wet conditions when germination is slow. This susceptibility to pre-emergence failure can be detected by soaking seed in deionized water and measuring the amount of electrolyte leached out by determining the electrical conductivity of the water. Samples which release large amounts of electrolytes give a high reading and may not be suitable for early planting; if very high values are obtained the seeds may not be suitable for planting at any time.

As is the case for many vigor tests, the leachate tests is not the ultimate answer in the search for the ideal vigor test and has not been accepted widely as a routine testing method. It is useful, however, if properly performed and interpreted, and has helped increase our understanding of seed vigor.