Seed Testing and Quality-Assurance

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High-quality seed is the basis for profitable and productive agriculture, be it a large commercial field operation, a small family-owned farm, or a greenhouse-based operation producing bedding plants or specialty horticultural crops. Assessing seed quality and assuring the buyer of the validity of the seed label (Fig. 1) has been an important mission of the various seed regulatory agencies of state and federal governments and service-testing provided by commercial seed-testing laboratories around the world. Based on validity of labeling, commercial sales of seed have been a profitable business to both the seed producer and the consumer.

In the United States and Canada, seed-testing rules have been developed and standardized by the Assn. of Official Seed Analysts (AOSA) and in other countries around the world by the Inter-

national Seed Testing Assn. (ISTA). The first state seed-testing laboratory in the United States was established in 1876 at the Connecticut Agricultural Experiment Station, and by 1930, 44 states had established seed-testing laboratories (Justice, 1961). In 1897, the first seed-testing rules in North America were prepared and published by the USDA in a circular entitled Rules and Apparatus for Seed Testing as unofficial guidance for seed analysts (Justice, 1961). The formation of the AOSA in 1908 and the passage of the U.S. Federal Seed Act in 1939 resulted in the formalized procedures for testing seeds that are acknowledged and used in the United States.

To meet the demands of new technology and new crops, the rules and procedures are reexamined and revised annually. For example, the development of seed-coating technology has led to changes and additions to the Rules for Testing Seeds as published by the AOSA (1988) and the International Rules for Seed Testing published by the ISTA (1985).

Assessment of seed quality includes tests for purity (physical and genetic), viability (germinable plus dormant), and vigor. Purity and germination normally are included as information on the seed label, along with a statement of the variety or cultivar. Vigor test results are not included as part of the regulatory labeling requirement. They are used extensively for in-house quality control by seed producers. A purchaser of seed may ask for vigor test results, or can obtain them from a commercial laboratory. Assurance of seed quality by a commercial producer involves continual monitoring of viability and/or vigor during all phases of harvesting, conditioning, and storage before the sale and shipping of the seed. Mechanical damage, seed contamination, and deterioration all must be avoided if high quality is to be retained.

Purity tests

The concept of seed purity includes both physical purity and genetic purity. Physical purity includes the presence or absence of soil, plant debris, weed seed, and other contaminants. Genetic purity relates to the presence of the correct cultivar and degree of contamination with other cultivars, or, in the case of hybrid seed, the presence or absence of self-pollinated inbreds or incorrect crosses.

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SEEDS

Lot No. _____
 Kind _____
 Variety _____
 Where Grown _____
 Locality _____

TEST

Pure Seed _____%	Germination _____%
Weed Seed _____%	Hard Seeds _____%
Other _____%	Total _____%
Crop _____%	G. + H.S. _____%
Inert Matter _____%	Date of test _____

Noxious Weeds

Name and number per pound of seed:

Seller _____
 Address _____

Fig. 1. Example of a seed label showing required information to be supplied to the buyer of the seed. Seed labels may vary somewhat from state to state. Note that vigor information is absent.

To determine physical purity, a random sample of the seed lot must be examined carefully by hand, often using a microscope. Each seed is observed by a trained analyst. This test is very tedious and time-consuming, but is necessary to evaluate the degree of contamination by noxious weeds, common weeds, and other crop seeds.

Genetic purity is determined either by morphological characteristics of the seed or seedling, or by using various chemical tests (Table 1) such as the fluorescence test and the phenol test (AOSA, 1988), both of which can be used to identify certain closely related cultivars or species. On a more sophisticated level, electrophoresis of seed proteins and enzymes can be used to separate cultivars that may differ by only one or a few genes. It should be stressed that in many instances a chemical test cannot identify an unknown cultivar; rather, the test can say what it is not. If it is known that the sample is either one cultivar or another, the test may make the distinction. More than one test often is needed to identify cultivars.

Viability tests

Seed viability normally is evaluated by means of the standard germination test. Although viability refers to seeds that are alive, in this test the term germination is used to count specifically those seeds that are capable of producing normal seedlings. A representative sample of at least 400 seeds (four replications of 100 seeds) is planted on special blotter paper, germination toweling, in sand, or in some other suitable substrate. The sample is placed in a germinator under controlled conditions of temperature, light, and water, and is evaluated after 7 to 21 days (or longer, depending on the species being tested) for germinability. Seedlings are classified as being normal or abnormal (Fig. 2), depending on the presence or absence of all essential structures (roots, shoots, cotyledons, etc.). Descriptions of what constitutes a normal or abnormal seedling is an integral part of the seed testing rules. Seed analysts are trained in interpretation of these descriptions and often participate in seed schools or in referee tests, which contribute to standardization among various laboratories of these subjective analyses. The Society of Commercial Seed Technologists requires new commercial analysts to complete a 2-year training program and pass a rigorous written and practical test before conferring the title of *Registered Seed Technologist*. The AOSA has a similar program of training and testing that leads to certification of seed analysts.

When dormancy is encountered or when a quick test is desired, the *topographical tetrazolium* (TZ) test can be employed. Depending on the species, seeds are placed into TZ solution either as whole seeds or after being bisected with a scalpel. Whole seeds are placed in 1.0% TZ solution and bisected seeds are placed into 0.1%

TZ solution. Staining time varies from 0.5 to 8.0 h, depending on the seed preparation. The colorless TZ solution is taken up by the seed tissue and, if dehydrogenase enzyme activity is present (indicative of cell viability), the TZ solution is reduced to form an insoluble red stain in the respiring tissue. This red color then is quantified subjectively and related to seed viability. This test also is used to detect seed vigor (see below).

Another test that can detect empty seeds, shriveled embryos, and/or insect damage is the *X-ray test*. Initially used for tree seeds (which have a long germination period), the X-ray test can examine a seed lot within a few seconds or minutes and reveal information about potentially dead or empty seeds. The seeds are placed directly over the X-ray film (or Polaroid film) and exposed to a brief dose of soft X rays. After developing, the film is read directly to interpret damaged or abnormal seeds.

Vigor tests

Several vigor tests have been developed over the years to measure seed performance under a wide range of field conditions. These tests also may be applicable to predict seed storage (Roos, 1989). The basis for vigor testing is the assumption that seeds undergo a sequential loss of cell function, which culminates in the loss of germinability (Fig. 3). While this scheme provides a simplistic illustration of what is thought to occur, the exact sequence of events is not known. Exact procedures for conducting most of these tests have been summarized in the *Seed Vigor Testing Handbook*, published by AOSA (1983).

Accelerated aging is one of the most widely used tests for predicting storability and field performance, as it can be applied to a wide range of species. Seeds are exposed to very high

Table 1. Chemical and other tests used for identification of cultivar purity (AOSA, 1988).

Type of test	Cultivar or species
Copper sulfate-ammonia Fluorescence	Yellow vs. white sweetclover. Distinguishes oat cultivars, hard vs. red fescue, annual vs. perennial ryegrass.
Hydrochloric acid Peroxidase	Distinguishes oat cultivars. Distinguishes soybean cultivars.
Phenol	Distinguishes among cultivars of barley, bluegrass, oats, ryegrass, and wheat.
Postassium hydroxide	Determines presence of red rice seed, distinguishes sorghum cultivars.

Fig. 2. Illustration of abnormal and normal seedlings of bean (*Phaseolus vulgaris* L.). (A) Loss of apical meristem. (B) Transverse cracking of cotyledons. (C) Broken hypocotyl. (D) Normal seedling.

humidity (95% to 100%), at a temperature of 40 to 45C (specific temperatures vary for different species), for a period of 2 to 4 days (again, specific time periods vary for different species). At the end of the aging period, seeds are subjected to the standard germination test and the results are compared with control (unaged) samples from the same seed lot. The chief difficulty in getting repeatable results is due to nonuniform uptake of water during the exposure to high humidity.

Controlled deterioration (Matthews, 1980) is similar to accelerated aging, except that seeds are first partially imbibed on moistened filter paper to predetermined moisture levels (thus avoiding the problem of uniform water uptake). Moisture content is checked by frequent weighing during this imbibition. The seeds then are sealed in containers and held for 24 h at 10C to

equilibrate before being incubated in a 45C water bath for 1 day. Seeds then are germinated (until radicle emergence) at 20C and compared with unaged controls.

The cold *test*, which is used primarily for predicting field emergence of maize seed, is used routinely by major seed companies for their evaluation of seed lots. This test was developed in the 1940s and involves the use of soil, taken from the field, that contains various microorganisms. Seeds are planted in plastic boxes containing the appropriate substrate and moisture and held at 10C for 7 days and then transferred to another chamber at 25C. Seedling counts are made 4 days later.

The *cool germination test* was developed for sensitive species that could not tolerate the low temperature of the cold test. Soil is not used, and the

temperature is a constant 18C: otherwise, the test is identical to the standard germination test. This test was developed specifically for testing cotton seed lots.

The *conductivity test* measures electrolytes leached from the seed during water uptake. It was proposed initially as a seed vigor test for predicting field emergence of peas. The principle of the test assumes that seed deterioration is manifested by loss of cell membrane integrity. If this assumption is correct, this test should have wide application to different species.

Seedling growth rate is simply a comparison of root and/or shoot length or seedling weight after germination (under controlled conditions and time period). Thus, it measures the metabolic efficiency of mobilization of storage reserves by the seed. It

Fig. 3. Probable sequence of changes in seed during deterioration (Delouche and Baskin, 1973).

is applicable to essentially all seed species; however, precise control of environmental conditions is needed to achieve reliable results.

The seedling vigor classification test is conducted in the same manner as the standard germination test. A further separation of normal seedlings is performed to identify strong and weak seedlings. Typically, weak seedlings may have no primary root (although secondary roots have developed), breaks or lesions in the cotyledons and/or hypocotyl, partial decay of the primary leaf, or are generally small and poorly developed.

Tetrazolium testing was developed originally as a quick estimate of seed viability, but can be used as a vigor test. The principle of the test is the measurement of nonspecific dehydrogenase enzyme activity, which results in a reduction of the soluble, colorless, tetrazolium salt to a red, insoluble, formazan that stains the respiring seed tissues. The TZ test can be used to assess vigor by critically evaluating the staining patterns of tissues. Although the TZ test has wide application, its subjectiveness makes it of questionable value for detecting small differences in vigor.

Quality assurance

The American Seed Trade theme is FIRST THE SEED, and to the horticultural crop producer that means the difference between harvesting a profitable crop or not. Quality assurance means different things to different people. For people working with

seed certification programs, it means being sure that the seed is inspected for genetic variability in the field, in the laboratory, and, after certification is complete, through grow-out tests. For seed analysts it means conducting tests to assure trueness-to-type, freedom from contamination, and the ability to produce normal, healthy plants in the field. Several state seed certification agencies have set up specialized programs for quality assurance and are providing this service to the seed producers.

Most quality assurance programs begin during seed production in the field. The use of high-quality, genetically pure planting seed, proper isolation distances, and weed- and crop-free soil are all beginning steps in a quality-assurance program. Using the proper seeding rates, row spacing, fertility levels, and irrigation scheduling is essential in producing high-quality seed.

A seed has its highest quality when it is physiologically mature. This stage of development usually is defined as that point during development when the seed has attained maximum dry weight. The quality of the seed at physiological maturity depends on the environment prevailing during seed development. The seed producer can control some of the environmental factors that affect quality, such as soil moisture, fertility, disease and insect stresses, and uniformity of stand.

Once the seed has been separated from the mother plant by the formation of an abscission layer, its quality is influenced by the environment, and deterioration begins. Crops such as beans (*Phaseolus vulgaris* L.) can be damaged easily during harvesting and handling. Factors such as moisture content during harvesting, mechanical damage during combining, and improper drying techniques all can lead to poor-quality seed. This damage can cause loss of vigor and/or viability, making the seed unusable for planting purposes. To prevent quality losses, seed companies have developed quality-assurance programs to evaluate, monitor, and minimize the loss of quality.

Quality assurance programs require systematic sampling and testing of the seed during production, conditioning, and storage. During seed production, a seed sample should be taken when the crop has reached physiological maturity. This sample is used to estab-

lish the level of quality and the seed moisture content before harvesting and conditioning. Once the proper moisture content has been reached for harvesting, another seed sample should be taken. This sample will indicate if seed quality has been lost during ripening. During harvesting, the seed should be checked for cracked or broken seeds to determine that the harvest equipment is properly adjusted. There are several quick tests that can be used in the field to check seed damage. Once the harvesting equipment has been adjusted properly, a sample should be taken to assess the quality after harvesting and to check seed moisture again. If the seed moisture content is not acceptable for temporary storage the seed must be dried before seed conditioning, preferably at low temperature to avoid loss of viability and vigor. After drying, a seed sample should be taken to determine if seed has been damaged during drying. Often seed is not conditioned immediately and is put into temporary bulk storage. Movement into bulk storage usually requires the use of an elevator, which can damage the seed, therefore, a seed sample should be taken to determine if quality is lowered.

Before conditioning, genetic purity tests should be conducted to determine if the seed meets the cultivar description. In most quality assurance programs the plants in the field were inspected to determine trueness-to-kind. However, cultivar purity tests of the seed will point out discrepancies during production that cannot be observed in the field. One of the common genetic purity tests that is conducted is a seedling grow-out. This evaluation is used routinely for vegetable crops. In addition to grow-out tests, many seed companies are now using electrophoretic fingerprinting. As cultivars become more specific in their environmental conditions for growth, these evaluations will become more necessary for a quality-assurance program.

Seed conditioning is an essential step in making seed of genetically superior cultivars available to crop producers. Some objectives of seed-conditioning are to remove contaminants, upgrade quality, improve plantability, apply seed treatment, and package the seed (Fig. 4). Each of the steps in this flow diagram requires specialized equipment that performs specific conditioning functions. However, as the seed passes through each piece of equipment,

Fig. 4. Basic flow diagram showing essential steps in seed conditioning (Vaughan et al., 1968).

it can be damaged. Consequently, a seed sample should be taken at each point along the way to assure that a piece of equipment is not reducing quality.

The work of a quality-assurance program is not completed when the seed is put into storage. If seed remains in storage more than a few months, it should be sampled and checked to determine if its vigor or viability has changed. It is also important that all storage areas be kept free of insects and rodents.

The quality-assurance program, as outlined, creates a large amount of data that can be helpful in determining where and why seed quality has been lost. When samples are evaluated using the various tests outlined above, a seed company can make the necessary modifications in their production practices, conditioning procedures, and storage facilities to produce and maintain a high-quality product, THE SEED.

Conclusions

The availability of high-quality seed does not just happen, but results from the ability of the seed analyst to determine the purity, viability, and vigor of each seed lot and the meticulous attention by the seed producer to overcome problems during seed development, harvesting, conditioning, and storage. Seed testing has become a highly technical field that requires specialized knowledge and training. New tests for genetic purity (cultivar identification) and vigor are under development and will require sophisticated instruments and highly trained analysts.

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