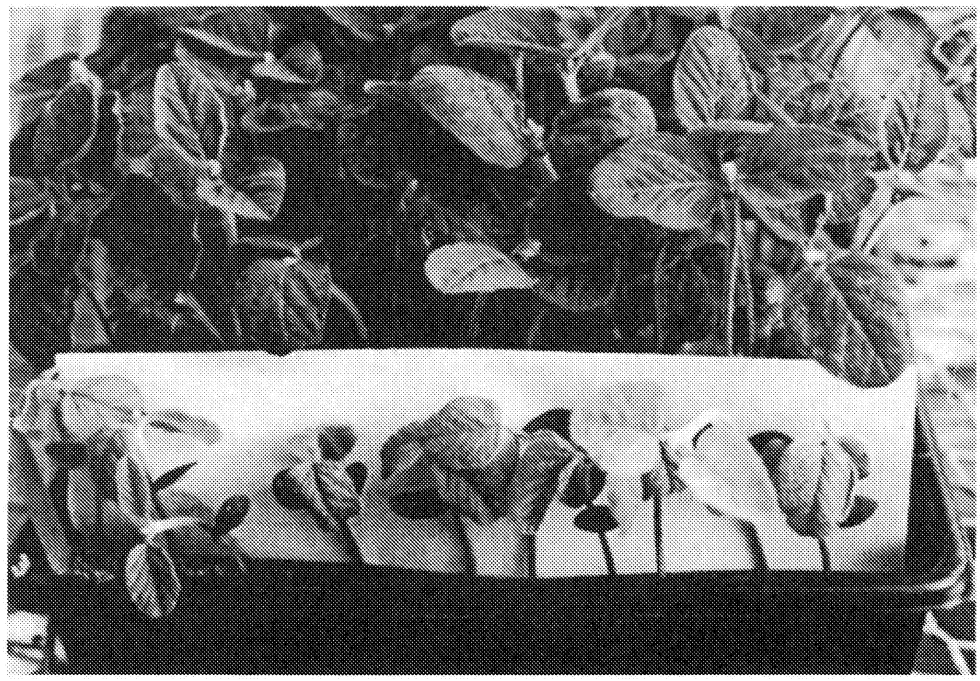


Figure 15.14. Seedling spray tests conducted in a greenhouse on Roundup Ready soybeans. Seedlings in the front are non-trait and susceptible to the herbicide while seedlings in the back possess the tolerance trait and are not susceptible to the herbicide (Courtesy of Illinois Crop Improvement Association).

Enzyme Linked Immunosorbant Assay (ELISA). Antibodies are substances that recognize the unique molecular structure of proteins or antigens and then bind to them. These antibodies are very specific and bind only to specific types of proteins. Thus, if a corn seed has the Bt gene for insect resistance, it will produce a specific Bt protein that an appropriate antibody binds to. However, since the bound antibody and antigen are too small to visualize, an enzyme label is added to the mixture that generates a visible color change in a substrate if the enzyme successfully combines with the antibody/antigen complex (Figure 15.15). The color reaction for each seed can be read by eye in 96-well microtiter plates. Commercially available ELISA kits and reagents are available for genetically modified crops such as Bt corn and Roundup Ready™ soybeans.

Electrophoresis. Electrophoresis of seed proteins/enzymes is a highly versatile approach to genetic purity testing of seeds–so much so that this technology has been incorporated into the Rules of the International Seed Testing Association and has been described in the Association of Official Seed Analysts' Cultivar Purity Testing Handbook (1991). Most electrophoretic systems employ either starch or polyacrylamide gels as the preferred media in which protein separations based on molecular size and charge density are made. It produces a separation of seed proteins or isoenzymes in the media by establishing an electric field and permitting the proteins to arrange themselves according to their polar (positive or negative electrical charge) nature; those with a more positive charge will align themselves near the positive pole. After the protein pattern has been established, it can be photographed and compared with the patterns of

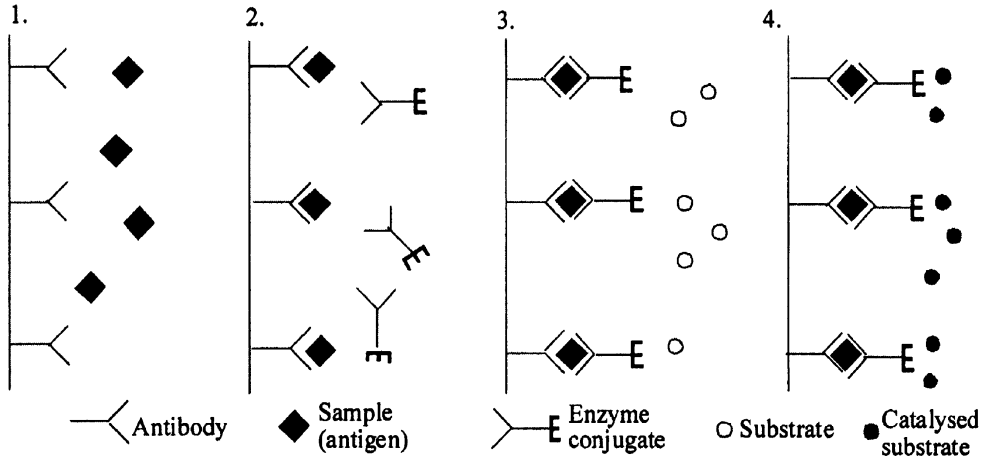


Figure 15.15. Diagram of a double antibody sandwich ELISA test (Courtesy of Ag Dia, Inc.).

known varieties. Another approach is the use of isoelectrically focused gels that separate proteins based on their position within a pH gradient; the protein ultimately coming to equilibrium at a pH where the molecule is no longer charged (isoelectric point or pI). All of these systems possess advantages and disadvantages from a commercial perspective (Table 15.1). Principal among these is the ability to evaluate sufficient numbers of seeds at the lowest cost. Starch gel electrophoresis systems have been described that allow adequate numbers of seeds (usually 80) to be run at one time (Stuber et al. 1988). More importantly, starch gels can be molded that permit up to five slices of the parent gel, each gel slice being evaluated for a different enzyme staining pattern (Figure 15.16). This system enables the equivalent of five electrophoretic evaluations of the same seed samples, thereby significantly reducing costs and analytical time. Starch gel electrophoresis of maize seed proteins provides reproducible and standardized results within and among seed testing laboratories and is commonly used in quality control programs in the maize seed industry (McDonald 1990). Other approaches have been identified to make electrophoresis more efficient and cost effective. One technique is to miniaturize and computerize the electrophoresis process. This results in less time being committed to electrophoresis, staining and destaining of gels. Such an approach has been developed, evaluated and found commercial application (McDonald and Drake 1990). Another strategy is to utilize non-denaturing isoelectric focusing gels that cannot be sliced and blot the proteins from the parent gel onto a nitrocellulose transfer membrane. This blotting technique allows multiple enzyme staining patterns from one electrophoretic run (Figure 15.17) and provides greater resolution of banding patterns than obtained on a starch gel (McDonald 1991).

Table 15.1. Advantages and disadvantages of polyacrylamide, starch, and IEF (isoelectric focusing) electrophoresis for genetic purity testing of seeds (McDonald, 1995).

	Polyacrylamide	Starch	IEF
Advantages	Technical system well defined	Technical system well defined	Technical system well defined
	Excellent band resolution	Inexpensive	Uses charge (pI) rather than charge density and size of proteins
		Gels can be sliced	Gels can be blotted
			Short running time (1.5 h)
Disadvantages	Expensive	Standardization of gels	Expensive
	Cannot slice gel	Long running time (5-6 h)	Cannot slice gel
	Potentially toxic	Poor band resolution	

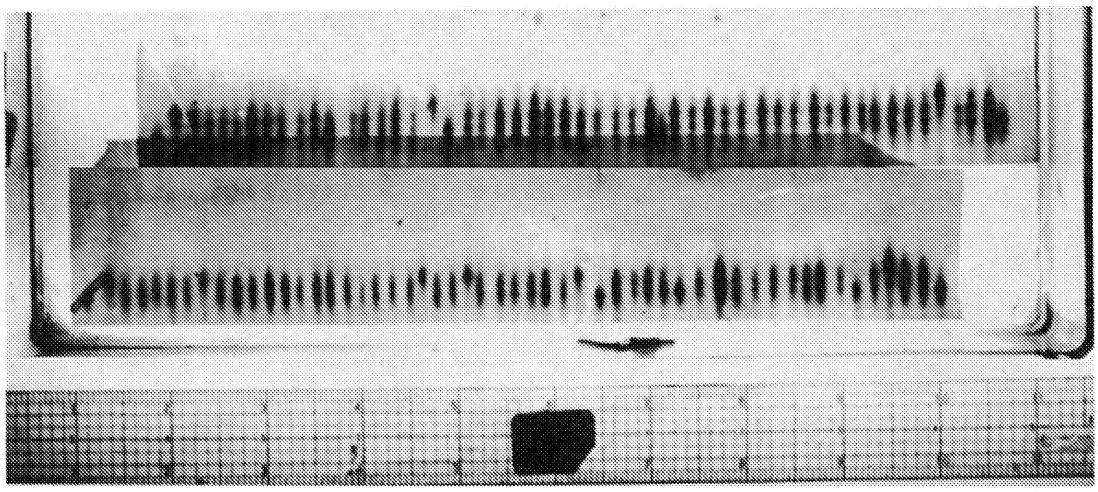


Figure 15.16. A starch gel stained for malate dehydrogenase isozymes. The banding patterns appear vertically on the gel as lines. Each line represents isozymes from a single maize seed (Courtesy of Novartis Seeds, Inc.).

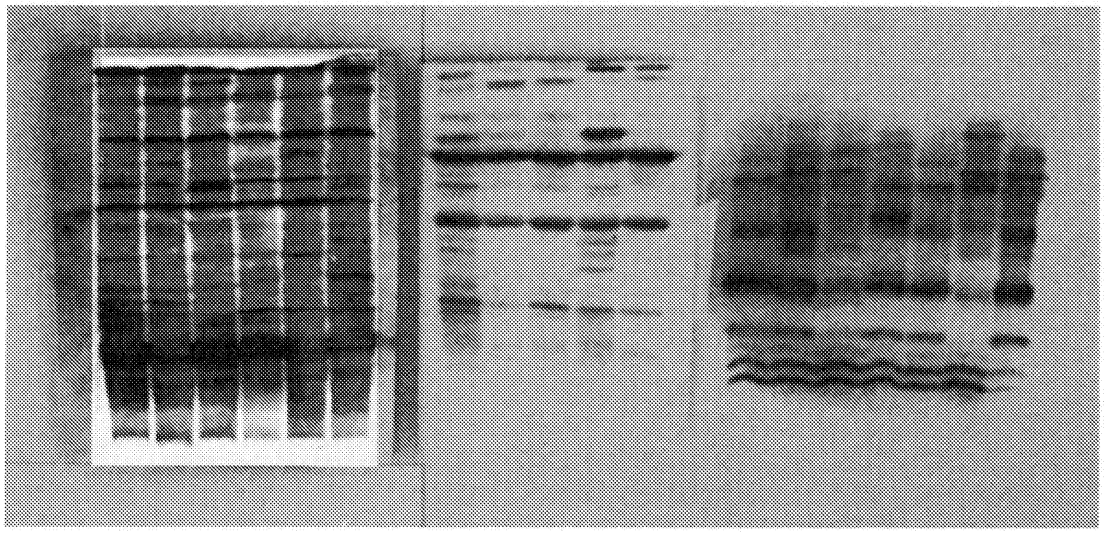


Figure 15.17. Blotting of oat cultivar seed proteins assayed for total protein (left) on the parent isoelectrically focused gel with blotted membranes demonstrating peroxidase (middle), and esterase (right) enzyme activity (McDonald 1991).

Polymerase Chain Reaction. Still, these techniques fail to differentiate a number of varieties in some crops. Greater sensitivity in genotypic identification is desired. In 1990, a new genetic assay called random amplified polymorphic DNA (RAPD) based on the uses a single arbitrarily chosen oligonucleotide primer that hybridizes to the genomic DNA template at two different sites, one on each strand of the complementary DNA. Under appropriate temperature alternations, a thermostable DNA polymerase is able to synthesize discrete DNA products (usually 200 to 2,000 base pairs long) that can be resolved on an agarose gel following electrophoresis. Each primer has the capability to consistently direct amplification of several unique DNA fragments in the genome. Some amplified fragments or patterns of fragments may be unique to a genotype (Figure 15.18; Jianhua et al. 1996) and hence useful in genetic purity testing. It should be emphasized that this area of seed technology is rapidly advancing and the development of more robust and standardizable genetic purity tests based on DNA technologies is in the immediate future.

Other Cytological Methods. Although other cytological methods have not been used extensively in genetic purity testing, they offer considerable potential. Cytological testing methods should become more valuable as new ways are found to introduce and direct chromosomal aberrations for creating new plant varieties having new predetermined characteristics (for example, disease resistance). In such instances, the presence of known chromosomal aberrations, such as deficiencies, duplications, inversions, and translocations, might be used for positive varietal identification.

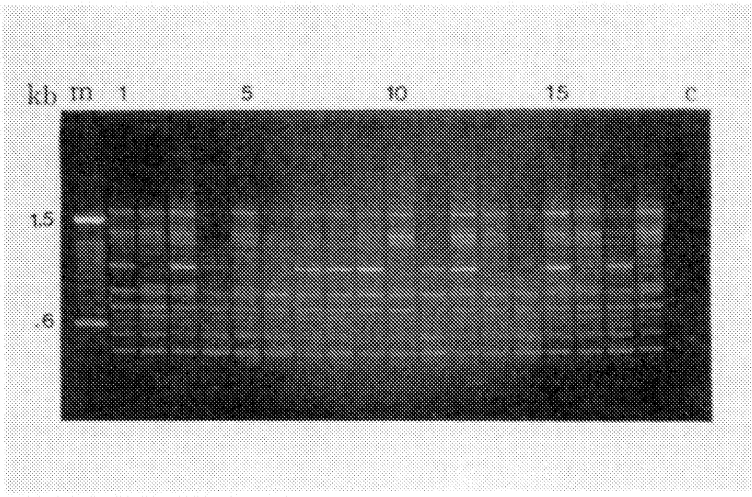


Figure 15.18. RAPD polymorphisms with primer 244 for 18 soybean cultivars. M is the base pair lane (Jianhua et al. 1996).

Disease Resistance for Varietal Identification. A pathogen inoculation test can be easily used to distinguish between disease-resistant and disease-susceptible crop varieties that otherwise appear similar. The test has been successfully used to distinguish between phytophthera- resistant and susceptible soybean varieties (Figure 15.19) and also in corn hybrids with different responses to southern corn leaf blight – for example, Texas male sterile varieties versus normal cytoplasm varieties.

Determining the Effectiveness of Seed Treatments

This test is used to determine the effectiveness of seed treatment with chemical pesticides. Although state and federal laws require treated seed to be dyed a contrasting color (see Chapter 18), the effectiveness of the coloration does not indicate the completeness and effectiveness of the treatment. The common method of testing the effectiveness of fungicide treatment is to plate treated seed on agar media and apply a covering of *Gibberella, Glomerella,* or *Cingulata* spores over the entire media surface. If seeds are ineffectively treated, the spores should germinate and grow around the seeds. A clear zone soon appears around each effectively treated seed where spore germination is prevented (Figure 15.20).

Effectiveness of Inoculation of Legume Seed

Tests for effectiveness of legume seed inoculation can be performed by the grow-out of seeds in the greenhouse or in growth chambers and their comparison to well-inoculated control samples. This test has been routinely performed in Indiana, and the information obtained used in the enforcement of that state's seed law pertaining to preinoculated seed (Figure 15.21).

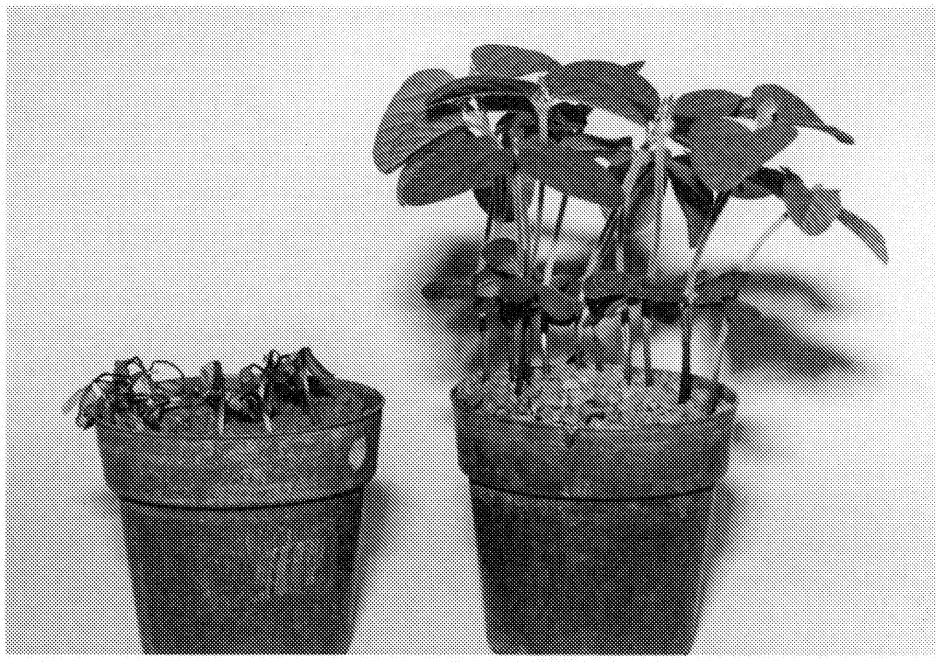


Figure 15.19. A test for resistance to Phythphthora root rot in two soybean varieties. The one on the left is susceptible (Courtesy of A. F. Schmitthenner).

Seed Moisture Test

Evaluation of seed moisture content is an extremely important determination in seed testing. Knowledge of the seed moisture content is useful because it provides information regarding the potential for harvesting, cleaning, and planting injury(ies) as well as the likelihood for successful long-term storage. A seed moisture test is conducted by first weighing the seeds to determine their "wet" weight. Then, the seeds are placed into an oven set for 100°C for grass, legume, and cereal seeds or 85°C for tree seeds for 24 hours except for large or thick-coated pine seeds and oily seeds such as Brassica species in which 48 hours at 85°C is required. After the drying period, the seeds are removed from the oven, placed in a desiccator for 15 minutes to cool, and the "dry" weight of the seeds determined. Two methods are used to express seed moisture content and are calculated in the following way:

Wet Weight

$$\frac{\text{Weight before drying - Weight after drying}}{\text{Weight before drying}} \times 100 = \text{\% Moisture content (Wet weight basis)}$$

Dry Weight

$$\frac{\text{Weight before drying - Weight after drying}}{\text{Weight after drying}} \times 100 = \text{\% Moisture content (Dry weight basis)}$$

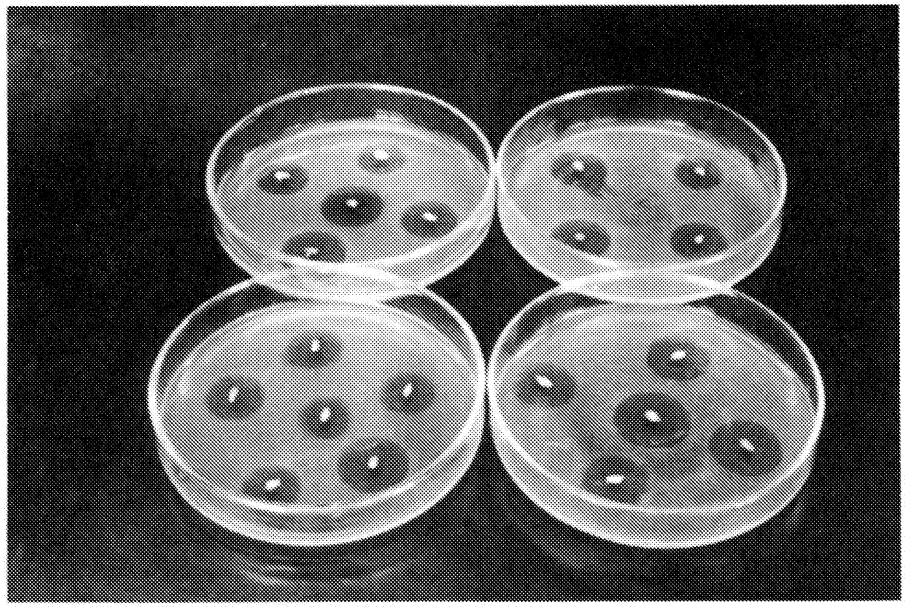


Figure 15.20. Inhibition zones around seed of an agar plating test indicate effective seed treatment.

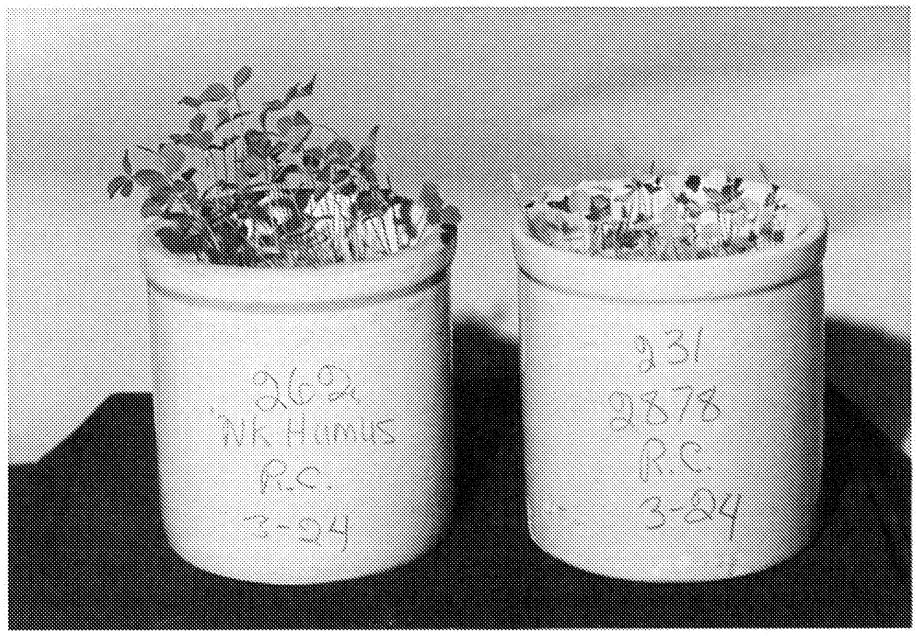


Figure 15.21. Left: plants from an effective humus inoculum applied to red clover seed at time of planting. Right: ineffective preinoculated seed (Courtesy of L. C. Shenberger).

In the seed trade, seed moisture is described on a fresh weight basis. The value will never exceed 100%. Research scientists often determine this value on a dry weight basis and the value can often exceed 100%. Thus, it is important that the analyst be aware of the specific procedure followed when percent seed moisture content is determined, since the calculations can lead to divergent results and interpretations.

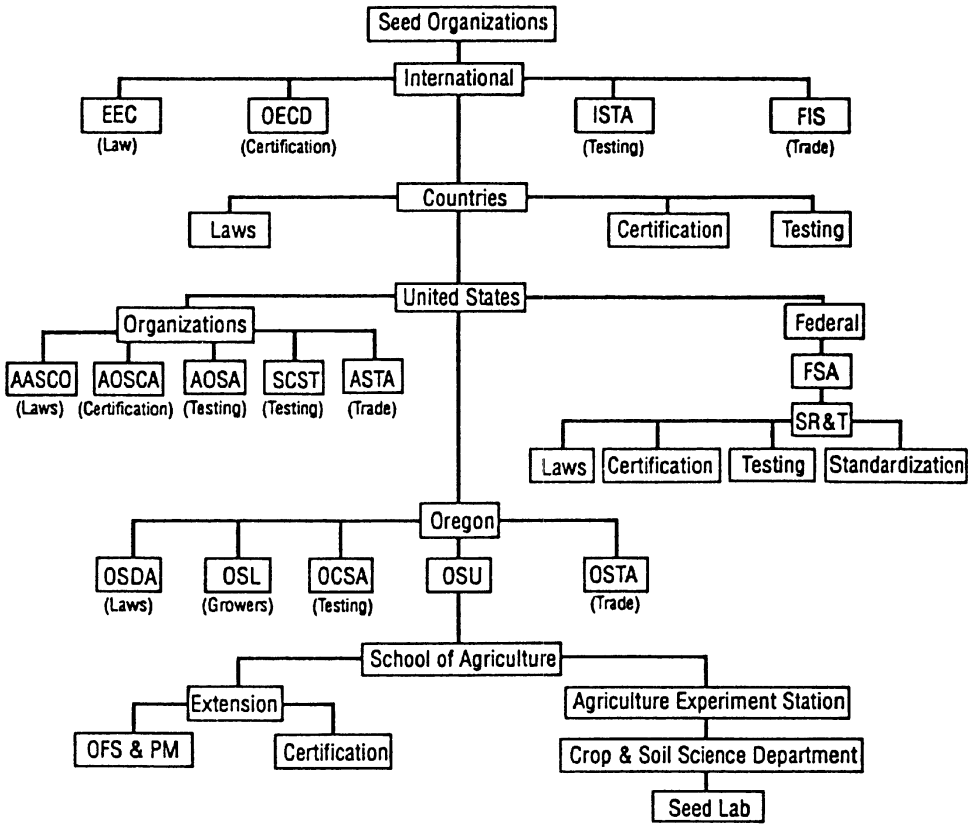
SEED TESTING TOLERANCES

The importance of having the working sample be representative of the entire seed lot can hardly be overemphasized since a nonrepresentative sample would provide erroneous results about the quality of the seed lot. Moreover, each time the working sample is reduced, determinations of the seed lot quality can tend to become less accurate. In addition, a seed sample is composed of individual biological units with their own inherent quality and performance characteristic, so it is not surprising that variability in test results between two working samples obtained from the same submitted sample would be obtained. Thus, variation in test results from the same seed lot is expected and normal. But, how much variation in test results is acceptable or tolerable? That is the purpose of seed testing tolerance.

Tolerances are used to define statistically acceptable limits within which different test results may be expected to vary. Tolerances have been established for the more common tests performed on seeds. They usually provide for the variability expected from random sampling error and some account for variability caused by interpretational errors or seed lot heterogeneity. Most studies of variation have shown that actual variability among different test results often exceeds that accounted for by existing tolerances. Because of this, seed law enforcement agencies sometimes allow "administrative tolerances" when determining if seed is improperly labeled. When properly applied, tolerances specify when results are "out of tolerance" or if a retest is necessary. Tolerances are based on the fact that the values reported have a probability of error of 5%. Tolerance is defined as the difference permitted between a labeled percentage (the first analysis) and the test results obtained by a laboratory when checking the accuracy of the labeled information. Tolerances for purity, germination, fluorescence, and noxious weed seed examinations are included in the Rules for Testing Seeds. The following example illustrates the use of germination tolerances. A state seed inspector picked up a Merion bluegrass seed sample from a lawn and garden store which was labeled as germinating 95%; an official germination test in the seed laboratory showed the sample to germinate 87%. To determine if the sample is mislabeled, both tests are given equal chance of being correct; thus they are averaged (95+87 /2) to give a weighted mean of 91%. The tolerance for the weighted mean of 91% is 6. The difference between the labeled germination and the second test is 8; consequently, the sample is out of tolerance and the seed lot is considered to be mislabeled.

SEED TESTING ORGANIZATIONS

The importance of seeds as an agricultural commodity is mirrored in the complexity of international, national, and state organizations concerned with assessing its quality (Figure 15.22). Many of these organizations are interested in the orderly movement of seeds from one country or state where the seeds are produced to the next where they are used. Of particular interest to seed analysts are those organizations devoted exclusively to seed testing. These include ISTA, AOSA, the Society of Commercial Seed Technologists (SCST), the Commercial



SELECTED SEED ORGANIZATIONS

AASCO - American Association of Seed Control Officials (Uniform Laws)
AOSA - Association of Official Seed Analysts (Standardize Testing)
AOSCA - Association of Official Seed Certifying Agencies (Standardize Certification)
ASTA - American Seed Trade Association
EEC - European Economic Community (Marketing)
FIS - Federation of International Seedsmen
FSA - Federal Seed Act (Regulates Interstate Seed Movement)
ISTA - Interernational Seed Testing Association (Standardize Testing)
OECD - Organization for Economic Cooperation and Development (International Certification)
RUSSL - Recommended Uniform State Seed Law (Standardize State Seed Laws)
SCST - Society of Commercial Seed Technologists
SR&T - Seed Regulatory and Testing

Figure 15.22. A typical flowchart illustrating important seed organizations at international, national and state levels (Courtesy of Oregon State University Seed Lab).

Seed Analysts Association of Canada (CSAAC), and the International Society of Seed Technologists (ISST).

International Seed Testing Association (www.seedtest.org)

The International Seed Testing Association (ISTA) is the only worldwide organization of national laboratories dedicated to seed testing on an international scope. Its goals include: (1)

development of rules for seed testing, (2) standardization of testing techniques, (3) seed research, and (4) cooperation with other international agencies for seed improvement.

The ISTA had its beginnings in the early 1900s, when seed technicians from several European laboratories felt the need for more exchange of seed testing information and communication among seed laboratories in different countries. During this period, the international seed trade was becoming established, creating the need for standardization of seed quality concepts across national borders.

This need was first put into action at the 1905 Botanical Congress in Vienna, during which several people met informally to plan a European seed testing association. Plans were made for a Seed Testing Congress in Hamburg, Germany, in 1906. Another Seed Testing Congress was held in 1910. Due to conditions in Europe, another meeting was not held until Professor K. Dorph Peterson, of Copenhagen, called a Third Testing Congress in Copenhagen in 1921, where the European Seed Testing Association was formed. Under the auspices of this group, the Fourth International Seed Testing Congress was held in Cambridge, England, in 1924. At this meeting, the name was officially changed to the International Seed Testing Association. Since its beginning, the ISTA has had great growth and accomplishments. It has become truly worldwide in both scope and representation. Membership in ISTA now includes 117 laboratories from 53 countries. Some of its notable accomplishments are:

1. In promoting uniformity of seed testing results among laboratories, it has facilitated movement of seed across international boundaries and helped farmers get the best possible seed regardless of the country of origin.
2. It has arranged for seed scientists and technicians to meet and discuss their problems and to find solutions for them. By drafting seed testing rules and by discussing their interpretations, they have provided a sound basis for enactment of seed laws to protect the farmer.
3. It has helped to achieve closer association between test results and field performance, assisting farmers to recognize seed of high planting value.
4. It has organized training courses and workshops in Europe, Africa, Asia, and South America to help promote seed testing in areas of rapidly emerging agriculture.
5. It has provided a focal point of seed knowledge.

The ISTA holds a Congress every three years at different locations throughout the world to hear scientific and technical papers from its members and to provide forums and committee meetings for the exchange of information and the finding of solutions to mutual problems. The complete activities at each congress are published in its official journal-*Seed Science and Technology* (prior to 1972-*ISTA Proceedings*).

Association of Official Seed Analysts (www.aosaseed.com)

The Association of Official Seed Analysts (AOSA) is an organization composed of analysts from official state, federal, and university seed laboratories throughout the United States and Canada. Its contribution in bringing seed testing to a respected and highly sophisticated level in these two countries has been enormous. Perhaps its greatest contribution has been the development of rules and procedures for seed testing, and the standardization of their interpretation. It also has had great influence on seed legislation in every state as

well as at the federal level. The Referee Committee of AOSA distributes problem seed samples to different laboratories for testing. Such activity helps attain standardization in procedures and interpretation among different laboratories.

The AOSA was formally organized in Washington, D.C. in 1908, with 16 states represented. Since its early days, the AOSA has held annual meetings almost every year. The minutes of its annual meetings and the papers presented are published in *Seed Technology* (formerly *Journal of Seed Technology; AOSA Proceedings*). It also publishes a newsletter in conjunction with SCST three times a year, which includes articles on seed testing topics. The Association has published many special publications, among which are a series of handbooks on selected topics.

Society of Commercial Seed Technologists (www.seedtechnology.net)

The Society of Commercial Seed Technologists (SCST) is a society of seed analysts from private or commercial seed laboratories throughout the United States and Canada. This includes self-employed seed analysts who test seed on a custom-fee basis and analysts from seed companies, who ordinarily are salaried or on a commission, and who test seed handled in the company's business.

The SCST originated in the early 1920s, largely as a liaison between the AOSA and the American Seed Trade Association (ASTA) because of their mutual need for better acquaintance and communication. The AOSA had regarded the ASTA suspiciously because they felt that some seed producers had flagrantly violated seed labeling laws and occasionally used fraudulent merchandising schemes. ASTA members regarded the AOSA as a well-meaning, but highly technical and regulatory-minded organization that promoted complex and often conflicting seed legislation. By 1922, some of the larger seed companies had their own seed testing laboratories and analysts. At the combined AOSA and ASTA meeting in Chicago in 1922, 13 commercial seed analysts met to form what was first called the American Society of Commercial Analysts. From the time SCST was first organized, there was good cooperation between the SCST and AOSA. These two organizations held their annual meetings at a common place, presented papers, exchanged ideas, and participated in referee testing together. The AOSA welcomed the new organization because it created a new bond of communication with the ASTA on a more technical and professional level. The respect for the SCST was strengthened by the high standards it established for society membership. In 1947, membership standards were further strengthened by the establishment of a comprehensive examination for membership. A minimum score of 80 was established for passing the test, which included: (1) seed identification, (2) purity and germination techniques, (3) evaluation of normal and abnormal seedlings, (4) knowledge of botany, (5) Canadian and United States federal seed laws, (6) official rules for seed testing and tolerances.

In addition, a combination of minimum college credit in the biological sciences and experience in seed analysis was established as a requirement. Analysts who pass all requirements and are accepted by a two-thirds vote by SCST members have the right to use the Society Seal and Insignia. The official seal is proof that the SCST member is a Registered Seed Technologist, and this becomes part of the analyst's credentials. Thereafter, the seal accompanies the results of any test performed under his or her supervision.

Commercial Seed Analysts Association of Canada (www.seedanalysts.com)

In 1944, six commercial seed analysts, formerly with the Toronto Seed Laboratory of the Canada Department of Agriculture, met in Toronto and formed the (www.cdnseed.org) Ontario Commercial Seed Analysts Association. The purposes of this organization were (1) to keep abreast of new methods of seed testing, and (2) to assist analysts in overcoming any problems that might arise in their work. Analysts from other Canadian provinces quickly showed an interest in this association, and at the second meeting in 1945, the name was changed to the Commercial Seed Analysts Association of Canada (CSAAC). The association has since grown to around 40 members, with representatives from Ontario, Alberta, Quebec, the United States, and England.

The Association holds its annual meetings in Toronto, and proceedings of this meeting are published in the Maple Leaf, the official publication of CSAAC.

Most members of the GSAAC are also members of the Society of Commercial Seed Technologists, so close communication is maintained between these two organizations. Members of CSAAC also attend meetings of the Association of Official Seed Analysts.

International Society of Seed Technologists (ISST) (www.seedtest.org)

The International Society of Seed Technologists (ISST) was formed in 1997. Its purposes are to maintain and encourage the highest proficiency and professional standards among its members; to promote seed technology research, teaching and extension activities; to promote improvements in seed testing rules and procedures; to promote the best interests of domestic and international seed trade; and to encourage cooperation between regulatory and commercial agencies. The organization of ISST is based on the establishment of world-wide chapters composed of seed technologists from differing countries. While ISTA is composed of government-supported regulatory seed testing laboratories, ISST's emphasis is in support of seed technologists around the world.

Questions

1. Do you consider seed testing to be a science, a skill, or an art?
2. What four components are considered to be part of the purity separation?
3. Define a noxious weed. How is the incidence of noxious weed seeds recorded in the purity test results? Why is a larger seed sample examined for noxious weed seeds than for the purity test?
4 Explain the normal seedling concept in interpreting laboratory germination tests.
5. What is the difference, if any, between hard seed and firm ungerminated seeds?
6. List several ways of stimulating faster germination in the seed-testing laboratory.
7. Describe several ways of distinguishing among varieties in a seed testing laboratory. Which do you consider to be the most practical in routine seed testing? The least practical?
8. Do you feel there should be more emphasis on pathological testing in the United States and Canada? Can more emphasis be justified in view of its costs and results? If so,which kind of pathological tests should be used? Which are presently in use? (See Chapter 16).

9. Do you think most seed samples submitted by seed growers for testing are properly drawn? Do you believe a grower would ever purposely misrepresent a sample to make a seed lot appear better? How many bags should be sampled from a seed lot containing 250 bags of seed?
10. What is the difference between sampling and subsampling?
11. What are the purposes of tolerances? Do you know how they are applied for the various test results?
12. Name several seed testing organizations. What is a Registered Seed Technologist?
13. Define a variety and identify two principal reasons why genetic purity testing is important.
14. List four advantages of performing genetic purity tests.
15. What are the criteria for a successful genetic purity test?
16. What are some disadvantages of field testing for genetic purity?
17. Describe three types of morphological tests for genetic purity.
18. Explain the advantages and disadvantages of presoak, substrate imbibition and seedling spray herbicide tolerance tests.
19. Identify at least three different electrophoretic approaches that can be used in genetic purity testing.
20. What are the specific advantages of polymerase chain reaction technologies for genetic purity testing?

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