INTRODUCTION TO SEED TESTING

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Seeds, soil, and climate are the basic ingredients of agriculture. It has long been a consensus that the more that is, or can be known about these prime ingredients, the greater will be the probability of an efficient and productive agriculture. Man selects crops adapted to predominating climatic conditions of his area and can - to a limited extent - alter climate to meet particular requirements of crops (e.g., irrigation, drainage, flooding). Within recent years soil resources have been surveyed and characterized in many parts of the world. Soil testing laboratories evaluate certain significant chemical and physical characteristics of soils. Upon the basis of such tests, fertilizer and soil management practices are recommended to the farmer. It is not surprising then, that seed testing has also become a standard agricultural practice.

There are several reasons why it is necessary (or desirable) to test seeds. The first, the most obvious, and the most important reason is to determine the quality of seed. By seed quality we mean the level or degree of their suitability for a particular purpose - producing a crop. In those countries having laws regulating the intra-country or inter-country (import) movement of seed, another reason for testing seeds is to fulfill certain legal requirements. A third reason for testing seed - which is closely related to the first - is to provide a basis for price, and consumer discrimination among the several seed lots available for purchase.

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Seed Quality Attributes

The question might be asked - for what does one test seeds? Seeds are tested for certain attributes which significantly affect their suitability for planting. These attributes are:

1. Physical purity
2. Incidence of noxious weed seed
3. Germination or viability
4. Provenance or origin
5. Density (weight per volume or number)
6. Moisture content
7. Varietal purity
8. Vigor
9. Incidence of seed borne diseases
10. Efficacy of various seed treatments
11. Homogeneity

Standard Seed Tests

Not all of the eleven attributes of seed quality listed above are evaluated routinely. Indeed, only three of them are routinely evaluated in most seed testing laboratories: physical purity, incidence of noxious weed seeds, and germination. To the extent that provenance and varietal purity can be evaluated in the purity test, then they too are routinely considered.

Purity

The physical purity of seeds is evaluated and determined in the purity test. The purpose of the test is to determine the physical composition of the seed lot (a lot of seed can be defined as a quantity of seed uniform in all its parts for the various seed attributes within certain tolerance limits). The test is made upon a seed sample of prescribed minimum size which is representative
of the lot. The quantity of seed prescribed for purity tests is based on a 3000 to 5000 seed minimum. Under certain rules, the purity sample is divided into two subsamples for independent analysis. Under other rules, the purity sample is examined in toto.

The purity sample is separated into four components:

1. **Pure Seed** - refers to the kind or kind and variety under consideration.
2. **Other Crop Seed** - refers to other kinds of crop seed or varieties other than the kind constituting the pure seed.
3. **Weed Seed** - refers to seeds or other propagating structures found in seed of plants recognized as weeds by law, regulation, or general usage.
4. **Inert Matter** - refers to seed-like structures from both crop and weed plants and other matter not seed.

After the components of the purity test are separated, they are weighed and their proportion of the whole sample expressed as percentages.

**Noxious Weed Seed Examination**

The noxious weed seed examination is an extension of the purity test. The working sample (sample for test) is on the order of 5 to 25 times the size of the purity sample (except for large seed). In the noxious weed test, the incidence or rate of occurrence of certain weed seeds (usually designated by law or official regulation) is determined. The rate of occurrence is usually expressed as the number per gram (or ounce), or kilogram (or pound) of each kind of noxious weed seed found in the sample.

**Germination Test**

The germination test is a measure of the ability of seeds to germinate and produce plants. Seeds for the germination test are taken from the pure seed component of the purity test. Usually 400 seed are tested - in replicates of
4 x 100. The seeds are planted under prescribed conditions favorable for germination and early seedling growth, i.e., provided with moisture, a favorable temperature and a suitable substratum or other special requirement. Not only are the conditions of the test prescribed, but also the duration of the test in days. Usually, the germination test is evaluated at least two times (or more) during the test period. One of these is a final evaluation made at the termination of the test.

Germination tests are evaluated as follows:

(1) The number of normal seedlings are determined. Normal seedlings are those possessing morphological and physiological attributes indicative of their ability to produce a normal plant.

(2) The number of abnormal seedlings. Abnormal seedlings are those which for morphological or physiological reasons are incapable of producing normal plants.

(3) The number of dead seeds. Seed in which the processes of germination are not initiated because of excessive necrosis.

(4) The number of hard seed. Hard seed are generally alive - they do not absorb water because of water impermeability of the seed coat.

(5) The number of dormant seed (other than hard seed).

Although these observations are made, germination test results are traditionally reported in terms of % germination (% normal seedlings), and % hard seed (or non-hard dormant seed in some instances). Other observations made during the germination are important and should be noted: general vigor of seed; incidence of seed borne diseases.
Other Tests for Seed Quality

In addition, to the three routine tests of seed quality considered above, certain other tests are frequently made on seed. Some of these might be routinely done on certain kinds of seed or in certain laboratories.

Provenance or origin

It is sometimes important to know the origin of seed. Origin is particularly important in those crops such as Medicago and Trifolium, in which definite ecotypes exist, that is, types adapted to particular climatic conditions. Origin can often be determined by carefully considering (1) extraneous seeds (weed or other crop seed) present in the sample, (2) other materials (soil, insects) present in the sample, and (3) results of growing tests. The ISTA Rules prescribe certain procedures for determination of provenance or origin (see Section 11.0).

Density

Weight per volume or weight per 1000 seed tests are sometimes desirable as a measure of seed quality in the forage grasses and small grains (Triticum, Oryza, Hordeum, Sorghum, etc.).

Weight per 1000 seed tests are made either on air dry seed or oven dry seed.

Volume-weight tests are expressed as pounds per bushel or kilograms per hectoliter.

In general, the higher the volume weight, or weight per 1000 seed, the higher is general seed quality. Low volume weights are an indication of immaturity, insect damage, drouth effects, frost damage, and sterility.

Moisture content

The moisture content of seed has an important relationship to harvesting procedure and time, longevity of seed, susceptibility to insect and mold attack, and the extent and severity of mechanical damage.
Moisture content is determined by several methods:

(1) Air oven (105°C or 130°C)
(2) Distillation (toluene method)
(3) Electrical methods

Moisture content is expressed on a wet weight basis. Tests are usually made in duplicate.

Varietal purity

In some instances, varietal purity can be determined during the purity test. However, varieties of some seed kinds are so similar in appearance that special procedures based on close examination of a reduced number of seed or other methods have to be used.

Some of the special methods used in trueness-to-variety tests include:

(1) Ultraviolet analysis (Avena, Lolium)
(2) Phenol reaction (Triticum)
(3) Seedling color (Triticum, Glycine, Sorghum)
(4) Seedling characteristics
(5) Field or growth chamber tests.

Generally, the analyst proceeds with methods based on seed characteristics as far as they allow before undertaking the more specialized tests.

Vigor

Seed vigor is a difficult concept to verbalize. By vigor we refer to those attributes of seed, both morphological and physiological, which contribute to rapid, uniform emergence and growth of seedlings under a variety of field conditions. Vigor is a measure of the physiological stamina or "healthiness" – in a broad sense – of seed. Two lots of seed having the same germination may differ widely in vigor. One lot might completely fail in the field, while the other will produce a uniform stand rapidly.
Summary

Certain seed attributes and their relation to seed quality were discussed. The three routine seed quality tests, purity, noxious weed examination, and germination, were considered in detail. Other tests not in routine use, but which are most valuable in certain cases, were considered.
THE GERMINATION TEST
An Introduction

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The germination test is a measure of the ability of seeds to produce normal plants. The germination test is usually made under the most favorable conditions for each kind of seed. Since the different kinds of seed vary in their optimum requirements for germination, specific methods and procedures for germination testing have been prescribed in the Rules for Seed Testing. For the most part, the methods and procedures prescribed are based on research findings.

The Germination Process

The germination process can be characterized in terms of three separate but not always distinct phases.

Absorption of moisture

The first phase of the germination process is absorption of water. Absorption of water is necessary for hydration of cells to provide a suitable environment for accelerated metabolic activities. The seeds of cereals and other Graminae require moisture contents of about 30 to 35% for germination. Seeds of Leguminosae or other types in which the principal food reserves are stored in cotyledonary tissue, require moisture contents in the range 50-55% for germination.

The moisture absorbing power of seed is considerable, so that they will germinate under rather dry conditions. With an optimum moisture supply, the seeds reach critical moisture contents for germination within 24 hours. Water absorption is a physico-chemical process and proceeds more rapidly at higher temperatures.
Food mobilization

As the level of hydration in the seed increases (and at a favorable temperature), certain enzymatic reactions are initiated. These reactions involve the transformation of complex, insoluble, non-translocatable food reserves (carbohydrates, lipids, proteins) to simpler, soluble, translocatable forms. The translocation of these soluble, simple compounds to metabolically active regions of the embryo and subsequent respiration provides the necessary energy and structural components for growth and differentiation.

Growth and differentiation

Growth of the embryo - the visible evidence of germination - involves both cell elongation and cell division. Cell division is usually initiated in the radicle about 24 hours after the beginning of water absorption. As the seedling develops, moisture content increases to about 85% (characteristic of succulent leaves and stems).

General Requirements for Germination

There are four general requirements for germination of most kind of seed. These are:

(1) Moisture
(2) A favorable temperature
(3) Sufficient oxygen
(4) Suitable substratum

Some seed analysts list a fifth requirement for germination, viz., light. Light, however, is more properly considered as a special treatment for overcoming seed dormancy in certain kinds of seed.

Moisture

A sufficient supply of moisture is necessary for germination. Moisture is usually supplied through the medium of the germination substratum. Excessive
wetting of the substratum or seeds should be avoided as it interferes with proper aeration. There is a rather specific minimum moisture content that seeds have to attain before the processes of germination can be initiated. This minimum moisture content ranges from about 30-35% for the cereals and small grains, to about 50-55% for legume seed and other seed in which the major food reserves are stored in cotyledonary tissue. Unless the water supply is heavily contaminated with the salts of heavy metals such as copper, zinc, and aluminium, it is not necessary to use distilled or de-ionized water for germination tests.

Favorable temperature

Seeds vary widely with regard to their specific temperature requirements for germination. For each kind of seed there are three cardinal points on the temperature scale: minimum, optimum, and maximum. The minimum temperature is the temperature below which germination will not occur. Sub-minimal temperatures seldom kill seed. The optimum temperature is the temperature at which maximum germination is obtained in the minimum amount of time. The optimum temperature is the one prescribed for germination of each seed kind in the Rules for Seed Testing. The maximum temperature is the temperature above which germination will not occur. Supra-maximal temperatures are usually lethal or at the very least cause thermal injury to the seeds.

Most kinds of seed of temperate climates germinate best at temperatures between 20° and 30° C. For these kinds of seed, the Rules prescribe an alternating temperature of 20° - 30° C., wherein, the lower temperature (20°) is maintained for 16 hours of each 24 hours period, and the higher temperature is maintained for the remaining 8 hours of the daily cycle. The 8 hrs. of high temperature usually correspond to the working hours of the laboratory (e.g., 8 a.m. to 4 p.m.)
The seeds of plants grown during the cooler seasons in temperate climates (Triticum, Hordeum, Trifolium, etc.) germinate best at a constant temperature of 20° C. Spinacia oleracea, a cool season crop, requires a temperature of 10° C.

Seeds of tropical or sub-tropical plants (warm season crops) are germinated under rather high temperatures of 30° or 35° C. constant temperature, or an alternating 20°-35° C. condition. Such seed include Paspalum spp., Lespedeza spp., and Alysiccarpus vaginalis.

Freshly harvested seed are generally more specific in their temperature requirements than are old seed. This response is related to residual dormancy.

For seed testing purposes germination temperatures must be accurately controlled - within an allowable range of plus or minus 2° C. from the desired temperature.

Oxygen

Oxygen is seldom limiting in germination testing unless excessive watering or moistening of the seed and substratum restricts aeration.

Suitable substratum

The functions of the substratum are to serve as a water reservoir and to provide a surface or medium on which or in which the seeds can germinate and develop. Various substrata are specified and can be used for germination tests. These include paper towels, paper blotters, filter paper, porous clay dishes, cloth, and sand or soil. The substrata should be reasonably sterile and in the case of paper or cloth, should be free of toxic chemicals. Possible toxicity of paper or cloth substrata can be checked by comparing germination responses of sensitive seed such as Brassica spp., Phleum pratense or other small-seeded grasses on the paper or cloth in question with those obtained on high quality filter paper. Failure of proper root development, or development of root galls or tumors, are manifestations of toxic effects.
During the germination test period, the substrata should be supplied with a uniform – but not excessive – amount of water.

Germination Testing Equipment

This topic is adequately covered in Agriculture Handbook No. 30, Manual for Testing Agricultural and Vegetable Seed, USDA.

Preparation of Germination Test

Seeds to be used for the germination test are taken from the pure seed component. The seeds are planted on appropriate substrata in replicates of 4 x 100 seed. In the case of large seed, it may be desirable to plant all 100 seed of each replicate on the same substratum. The replicate can be subdivided and planted on two sets of substrata at 50 seed per set. Regardless of number of individual sets planted, each replicate (100 seed) should be marked at the time of planting so that proper grouping can be made at the time of counting.

After planting, the seeds are placed at the temperature prescribed in the Rules for Testing Seed.

Evaluation of the Test

Usually two or more counts are made on germination tests. The time of the preliminary count is indicated (and should be considered as only approximate). During the preliminary count only "normal" seedlings should be removed and counted. In some cases it is also desirable to remove badly decayed or molded, dead seed or seedlings to prevent spread of the contamination.
HISTORY OF SEED-TESTING

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Although seeds have been the basis of agricultural development since the dawn of civilization, little attempt was made to evaluate their quality or regulate their movement until about 100 years ago.

In 1816, Switzerland passed a seed law requiring that at least two men in a county - where clover seed were sold - serve as inspectors of seed offered for sale. In 1869, the English Parliament adopted the Adulterated Seeds Act. That same year (1869), the first station for testing seed was established in Tharand in Saxony, Germany, under the direction of Friedrich Nobbe. (Nobbe's classic treatise on seed testing "Handbuch der Samenkunde" was published in 1876.) A few years later in 1871, a seed testing laboratory was opened in Copenhagen, Denmark, under the direction of E. Moller-Holst. At the beginning of the 20th century (1900), about 130 seed-testing stations were operating in Europe.

In the United States of America, the first law regulating the sale of seed was passed in Connecticut in 1821. However, it was not until 1876 when Dr. E. H. Jenkins, who had visited Nobbe's laboratory in Germany, established the first seed testing laboratory on the American continent at the Connecticut Agricultural Experiment Station. By 1900, seed testing was well established in the United States of America in the U. S. Department of Agriculture and in the states of Connecticut, Maine, Massachusetts, Michigan, New York and Vermont. Within 10 years (1910) at least 10 additional states were testing seed.

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Association of Official Seed Analysts

As seed testing developed it became obvious that procedures and methods for testing seed would have to be developed and standardized.

In 1908, a group of delegates from 16 states, the U. S. Department of Agriculture, and the Canada Department of Agriculture met in Washington, D. C., to consider standardization of seed testing methods and the framing of a model seed law. One significant result of this meeting was the formation of an organization then known as the Association of Official Seed Analysts of North America (the phrase "of North America" was dropped from the Association's name in 1939).

The first president of the AOSA was E. H. Jenkins of Connecticut. Annual meetings followed until 1917. In that year, rules for testing seed were adopted and published. Since 1917, seed testing rules have been periodically revised, and current complete revisions of the Rules for Testing Seed (AOSA) are published every five years.

The AOSA membership currently includes official laboratories from the various states of the United States of America, U. S. Federal Laboratories, several research laboratories, and laboratories operated by the Canada Department of Agriculture. The AOSA meets annually in various parts of the United States of America and Canada. Average attendance includes representatives from about 40 - 50 member laboratories. In addition to the Rules for Testing Seed, the AOSA publishes Contributions to the Handbook on Seed Testing, the News Letter (four issues each year), and the Proceedings (one issue each year) which contains committee reports, rule revisions, and technical papers. In 1959, there were over 750 analysts presently or formerly associated with member laboratories in the United States of America and Canada.
In addition to the AOSA, there are two other organizations in North America dedicated to the standardization and improvement of seed testing methods. These are: (1) The Society of Commercial Seed Technologists; and (2) Commercial Seed Analyst's Association of Canada. These two associations are composed of persons engaged in commercial testing of seed or associated with private laboratories of commercial seed companies. They meet annually and usually at the same time and place as the AOSA.

International Seed Testing Association

The history and development of ISTA will be considered by Dr. O. L. Justice in a separate discussion. However, it should be mentioned here that the first international meeting of seed analysts was held in 1905 in connection with the Botanical Congress in Wien. In 1921, the International Seed Testing Association was formed. The International Rules for Seed Testing were first published in English, German and French in 1931. They are periodically revised. The ISTA meets every 3 years.

Federal Seed Act (U.S.A.)

Although seeds were tested in the U.S. Department of Agriculture prior to 1939, it was in that year that a strong Federal Seed Act was adopted. Cooperative federal-state laboratories were immediately set-up in several states. There are presently six federal seed laboratories in the U.S.A. They act in cooperation with the various state seed laboratories in control of interstate movement of seed, and have jurisdiction over seed imported into the U.S.A.

The Federal Seed Laboratory, Beltsville, Maryland, has long been instrumental in arranging and conducting training courses in seed testing. These training courses have had a most significant influence on uniformity and standardization of seed testing in the U.S.A.
Summary of Significant Events

1816 - First seed law (Switzerland)
1821 - First seed law on American continent (Connecticut)
1869 - Adulterated Seeds Act (England)
1869 - First seed testing station established (Germany)
1876 - Publication of Nobbe's "Der Samenkunde"
1908 - Association of Official Seed Analysts organized (Washington)
1917 - Publication of first Rules for Testing Seed by AOSA
1921 - Organization of International Seed Testing Association
1931 - Publication of first ISTA Rules for Testing Seed
1961 - Designation of 1961 as "World Seed Year" by FAO