Seed Technology

Seed testing is an evaluation of seed quality parameters in order to ensure that the seed conforms to the minimum seed standards prescribed for the concerned seed class. Basically seed testing involves tests that are meant to verify the following three parameters: physical purity, genetic purity, moisture content and seed germination.

Seed science and technology concerns seed formation, development, seed sampling procedures, seed testing for physical analysis, identification, genuine to variety, moisture determination since seed is sold on a weight basis, these seed quality parameters are essentially also economic parameters. Moisture content in seeds is particularly important during storage, germination including substrates, pretreatments required (pre-drying-pre-chilling- scarification-chemical treatments-hot water) and test evaluation, rapid biochemical test of viability by tetrazolium chloride, vigor and seed health test.

The science of seed testing illustrates evaluating the planting value (agricultural value) of seeds has been developed to achieve the following objectives for minimizing the risks of planting low quality seeds:

1. To determine their quality and suitability for planting as the seeding rate based on seed purity and germination.

2. To identify seed quality problems, such as dormancy and their probable cause, treatments for breaking dormancy.

3. To determine the need for drying, processing and specific procedures that should be used for the damped seeds.

4. To determine if seed meets established quality standards or labeling specifications.

5. To establish quality and provide a basis for price and consumer discrimination among lots in the market.

History of Seed Testing

Organized seed testing started for long times as a necessary reaction to unscrupulous practices, prevalent in the seed trade during the nineteenth century. The first station for seed testing was established in **Thrandt, in Saxony, Germany, in 1869 under the direction of Friedrich Nobbe**. Nobbe's classic treatise on seed testing 'Handbuch der Samenkunde' (Hand Book of Seed Testing) was published in 1876. **A few years later in 1871, a seed stesting laboratory was opened in Copenhagen, Denmark, under the direction of E. Moller Holst.** Seed testing spread rapidly in Europe. At the beginning of the twentieth century (1900) about 130 seed testing stations were operating in Europe. In the United States, the first seed testing laboratory was opened in 1876. **The International Seed Testing Association (ISTA) was founded in 1924,** it became obvious that co-operation between seed testing stations was imperative for the establishment of common methods of testing that would secure uniformity in evaluation and test results. The primary objectives of (ISTA) are to develop, adopt and publish standard procedures for sampling and testing seeds, and to promote uniform application of them for the evaluation of seeds moving in the international seed trade. In addition, it also promotes research in all aspects of seed science and technology, including sampling, testing, storing, processing and distribution.

One of the foremost achievements of ISTA is the adoption of the International Rules for Seed Testing. These rules prescribe testing techniques based upon scientific evidence, which are accurate within stated statistical limits and practicable within the everyday operations.

The following objectives have served as guidelines (Agrawal, 1980):

1. To provide methods by which the quality of seed samples can be determined accurately.
2. To prescribe methods by which seed analysts working in different laboratories in different countries throughout the world can obtain uniform results.
3. To relate the laboratory results, in so far as is possible, to planting value.
4. To complete the tests within the shortest period of time possible, commensurate with the above mentioned objectives.
5. To perform the tests in the most economical manner.

Organizations of Seeds Testing

Association of Official Seed Analysts (AOSA) of North America, was founded in 1908, the first published rules were in 1917, and since then have been periodically revised every five years.

The **Society of Commercial Seed Technologists (SCST**) was organized in 1922.

The **Commercial Seed Analyst's of Canada (CSAAC). American Seed Trade Association (ASTA).**

In Iraq, the first seed testing laboratory was established in 1962 at the department of Field Crops-Ministry of Agriculture at Abu-Ghraib (Baghdad). With the end of 1967 it was joined to Seed Production and Certification Project. At 1970 it was joined to **Seed Testing and Certification and Gins- Directorate - General Secretariat.** At that time two stations were founded for this department- Northern seed testing and certification at Ninevah governorate which started to work at 1972, and Southern seed testing and certification at Dhi-Qar governorate which started to work in 1973. The department was then joined with **General Board of Seed Testing and Certification** governed by **General Commission of Applied Agricultural Researches** in 1979 (Ali, 19882).

In Kurdistan region the General Directorate of Seed Production and Certification was established in 2010.

Seed Sampling

Objectives:

The object of seed sampling is to obtain a sample of a size suitable for tests, which the same constituents in the same proportions as in the seed lot.

The seed sample tested in the laboratory is minute compared to the seed lot it represents (e.g., if 1g is analyzed from a 10.000 kg lot, the ratio is 1:10.000.000). To obtain uniform, accurate results in seed testing, it is essential that samples be prepared in accordance with the rules of the international seed testing association (ISTA). It is essential that the sample precisely represents the composition of the seed lot, and that the working sample obtained in the laboratory represents the submitted sample.

ISTA rules for sampling must be followed by seed testers, inspectors, and warehouse samplers, so the seed testing station authorized by ISTA can issue international green or orange certificates; otherwise, only the less valuable blue certificate can be issued.

Certificates:

Although various national certificates exist, three international ISTA certificates are important for the seed trade:

1 – **The orange seed lot certificate**, issued when sampling has been done according to ISTA rules by an official body approved by the seed testing station. The sampling and testing must have been carried out in the same country.

2 – **The green seed lot certificate**, with the same requirements as the orange, but with testing carried out by an authorized station in a country other than where the lot was sampled.

3 – **The blue seed sample certificate**, only referring to the sample. This certificate is used with unofficial sampling, when the seed testing station is not certain that the sample represents the seed lot. The certificate only refers to the quality of the sample received, and omits the name of the sampling and sealing agency as well as the lot's mark and seal.

Definitions:

Lot: A specified quantity of seed, physically and uniquely identifiable, for which an international analysis certificate be issued. The lot sizes and sample sizes has been fixed by ISTA Rules (2013).

Primary sample: A small portion taken from one location in the lot.

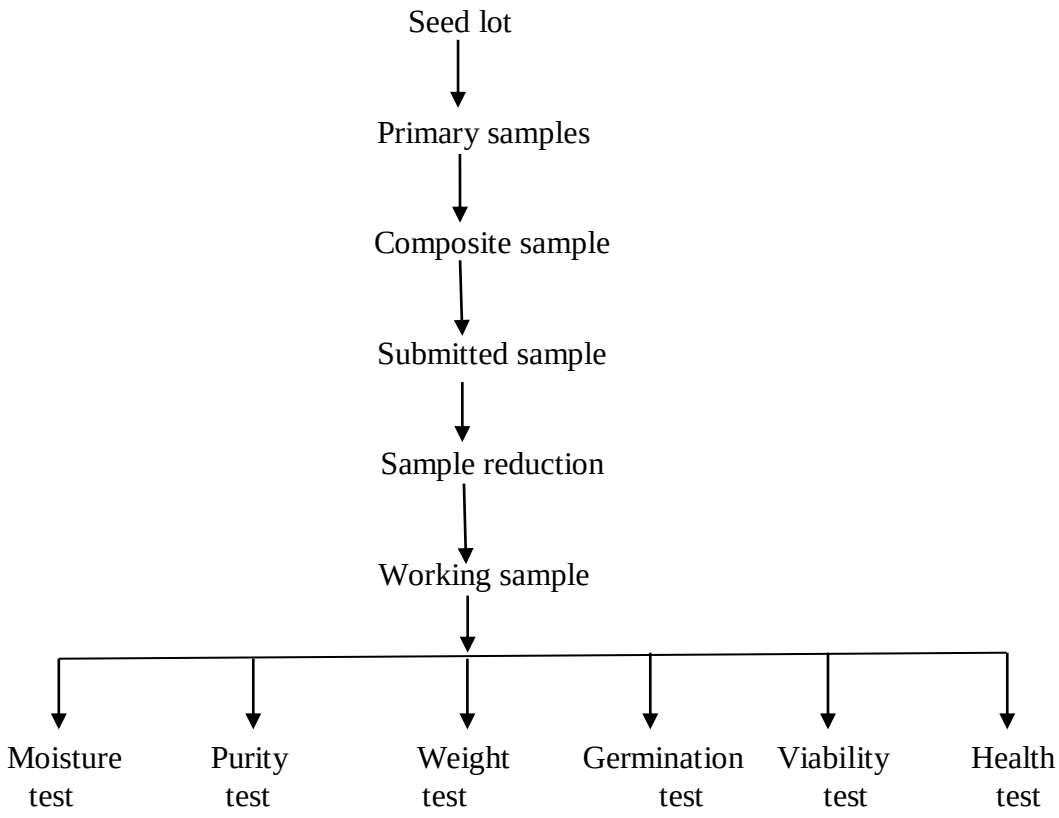
Composite or **Compound sample:**

Submitted sample: The sample submitted to the testing station, comprising the composite sample reduces as necessary (since the composite sample is usually much larger than tests require). It must be at least as large as the size specified ISTA rule 2.6.3

Working sample: A reduced sample taken from the submitted sample in the laboratory, used in a given quality test.

Sealed: A sealed container, for both lots and samples, is one that is closed in such a way that it cannot be opened and closed again without either destroying the seal or leaving evidence of tampering.

When seed lot arrives to the silo, it undergoes series processes as shown in the diagram below:



Principles and procedures for sampling the lot

A lot that is to be sampled must not show any heterogeneity, which means that all primary samples must be exactly the same in aspect. If there is evidence of heterogeneity (which can be confirmed with the heterogeneity test) sampling should not be carried out.

The size of the lot must not exceed certain limits. For most agriculture seeds the lot must not exceed 10.000 kg, but for large seeded species 20.000 kg is allowed. An exception is maize for which 40.000 kg is allowed. (Table 1, column 2).

Another sampling requirement is that the lot must be in sealed or sealable bags or other containers that are labeled or marked for identification with a single lot

designation. An international seed lot certificate cannot be issued for seed that is loose or stored in unsalable containers.

Table 1. Seed lot sizes and sample sizes for some field crops seeds.

Seed species	Max. Weight of Lot (kg)	Min. submitted Sample (g)	Min. working sample (g)	
			Purity Analysis	Other seeds by number
Brassica napus L.	10000	100	10	100
Cicer arietinum L.	30000	1000	1000	1000
Helianthus annuus L.	25000	1000	200	1000
Len culinaris Medik	30000	600	60	600
Nicotiana tabacum L.	10000	5	0.5	5
Oryza sativa L.	30000	700	70	700
Sesamum indicum L.	10000	70	7	70
Triticum spp	30000	1000	120	1000
Hordeum vulgare L.	30000	1000	120	1000
Vicia faba L.	30000	1000	1000	1000
Zea mays L.	40000	1000	900	1000

Methods of drawing seed samples

1. Seed Sampling Triers (**Probes**)

1.1. Sleeve or Stick Probe

Various instruments are allowed for sampling. A common instrument is the: **stick** or **sleeve-type trier**: consists of a hollow brass tube divided into a number of compartments inside a closely- fitting outer shell, or sleeve, which has a solid pointed end. When the slots in the tube and sleeve walls are aligned, seeds can flow into the tube cavity; a half-turn of the tube closes the openings. Tubes are designed for different kinds of seed and, various container sizes. In sampling cereal seed in bags, a ±760 mm trier with an outside diameter of ±25 mm and six slots is suitable.

1.2. Bin samplers

They are similar to the sleeve trier, but are much larger (up to 1.6 m in length and 3.8 cm in diameter, with 6 to 9 slots).

1.3. Nobbe or Theif Trier

Another type of trier, the **nobbe trier**, is suitable only for sampling bags. It is a pointed tube long enough to reach the center of bags, with an oval hole near the pointed end. The sampling should be varied from top, middle and bottom of the bags. A bag on the floor can be placed atop other bags to sample the bag bottom. For cereals, the distance from hole to handle should be about 350 mm and the internal diameter of the tube should be about 14 mm. the trier should be inserted gently into the bag.

2. Hand Sampling

In certain cases, and for certain species, especially chaffy, or seeds with outgrowth (wings) non-free flowing species, hand sampling is sometimes the most satisfactory method (e.g. Bromus, Cynodon, Festuca, Lolium, Panicum, Poa). It is difficult by this method to sample deeper than about 40 cm. It is requested to be emptied or partly emptied, to facilitate sampling. When sampling is done by hand, fingers should be tightly closed around the seeds so that seeds do not drop out.

3. Automatic Sampler for Seed Stream

Seed may be sampled by automatic sampling devices, provided that the instrument uniformly samples the cross section of the seed stream and the material entering the instrument does not bounce out again.

4. Pneumatic vacuum device

The sleeve trier can be attached with vacuum machine for drawing seed samples from vehicles.

For seed lots in bags of uniform size, there are minimum required sampling intensities. For 1 to 5 containers each container should be sampled and at least 5 primary samples taken. For 6 to 30 containers at least 1 in every 3 containers should

be sampled, but never fewer than 5. For 31 or more, at least 1 in every 5 containers should be sampled, but never fewer than 10.

Samples should be sent quickly to the seed testing station and never be left with the owner, applicant or others not authorized by the sampling agency or by the seed testing station.

Principle and procedures for laboratory sampling

Several methods and apparatus are used to reduce the submitted sample to the size of a working (Table 1, column 4):

Methods of dividing or reduction of seed samples

1. Hand Methods

For seeds of cotton and certain other species hand dividing methods of sub-sampling may be necessary.

Several hand methods are used:

1.1. Pie Method

It consists of spreading the sample on a clean, flat surface, and dividing it into sections as if cutting a pie. Any of the sections, if randomly selected, may be used alone or in combination with other sections as a working sample.

1.2. Random Cup

By placing a number of uniformly sized thimbles or cups on a clean flat surface and slowly pouring the sample so the seed is distributed evenly over the flat surface filling the cups as the seed is distributed. The working sample may then be obtained by normally selecting several of the cups until sufficient seed is obtained. The

method is suitable for seeds requiring a working sample up to 10 g., not chaffy structure and do not bounce or roll (*Brassica spp*).

1.3. Spoon Method

It is used for samples of a single small seed species. A tray, a spatula and a spoon with a straight edge are required. After preliminary mixing, pour the seed evenly over the tray, do not shake the tray thereafter. With spoon in one hand, the spatula in the other, remove small portions of seed from not less than five random places on the tray. Sufficient portions of seed are taken to constitute a working sample. Only spoon method and the hand halving method may be used in the laboratory to obtain working samples for seed health testing where other samples or equipment may be contaminated by spores or other propagating material.

2. Mechanical Divider Method

The submitted sample passed through in numerous slots, which portioned into various portions, and can be repeated many times and removing parts on each occasion.

Several instruments are used for these purposes – conical divider such as **(Boerner)** for large seeds, which consists of a hopper, cone, and series of baffles directing the seeds into two spouts.

3. Electronic Centrifugal Sample Divider (Gamet).

Storing the seed samples

The samples received in the laboratory should be tested as soon as possible to avoid moisture change. Storage if necessary should be in a cool, well ventilated room. Storage should be in accordance with the species requirements regarding temperature and humidity control, and protect against insects and rodents.

Seed Moisture Test

Objectives

Seed moisture content test must be done when seed lots arrive to determine whether it is within the allowable limit (13.5-14%) for orthodox seeds (exceeding this will rejected). It is important to know the moisture content immediately after harvest, prior to storage or shipment. Seed moisture content is one of the most important factors influencing retention of seed viability and general appearance of the seeds. Moreover, it has great relation with insects and pathogens infection. Later, during storage high moisture content decrease viability more rapidly because of mold growth, heating damage, aging and greater insect damage.

The moisture content of a sample is either the loss in weight, when it is dried, or the quantity of water collected when it is distilled. It is expressed as a percentage of the weight of the original sample on wet weight basis for trade purposes and of the dry weight basis for chemical analytical purposes.

It is important in trade, as it deals with the dry matter not water. Therefore, all chemical constituents must be converted on moisture content basis. The objective of moisture analysis is to determine the moisture content of a seed lot at sampling.

On the other hand, the grain moisture content has to be adjusted during milling process (conditioning or tempering) to around 15-17.5% which varies according to wheat varieties. During the determination the seed should be exposed to the laboratory atmosphere as little as possible.

Materials and instruments used for moisture testing

Grinding mill, sieves (mesh 0.05 mm, 1.0 mm and 4.00 mm), analytical balance accuracy ± 0.001 g, ovens, small aluminium can with lids, clamp, distillation apparatus, electrical moisture meters.

Methods of Seeds Moisture Determination

Basic Methods

Based on the removal of water from the seeds, when the dry matter is determined, it is assumed that no volatile material other than water was driven off. In all methods two samples must test, and the difference between the determinations **should not exceed 0.2 per cent;** otherwise repeat the determination in duplicate.

1. Drying without Heat (with desiccant)

In this method, the samples are dried without heat or with moderate heat in a vacuum, using phosphorous pentoxide (P_2O_5) as a desiccant.

2. Lyophilization (Freeze drying)

In this method, biological materials are frozen and the water **removed by sublimation in a vacuum.**

3. Titration method (Karl Fisher)

4. Distillation Methods

5. Air oven method

This is the most practical basic method of moisture determination in seed testing laboratories, and is included in the International Rules for Testing Seeds (ISTA).

For cereal and cotton seeds, fine grinding is necessary. At least 50 per cent of the ground material should pass through a wire sieve with meshes of 0.50 mm and not more than 10 per cent remain on a wire sieve with meshes of one millimetre. For leguminous and tree seeds, coarse grinding is necessary. At least 50 per cent of the ground material should pass through sieve with meshes of 4 mm.

Moisture Calculation in Oven Methods

Wet weight basis

The moisture content as a percentage of wet weight basis which is frequently used in research and in seed trade, the amount of water lost is divided by the initial (original) weight of the sample multiplied by 100, in the following formula:

Moisture content % (**wet weight basis**)= $\frac{\text{sample weight before drying}-\text{weight after drying}}{\text{weight before drying}}$ X 100

$= \frac{5-4.5}{5}$ x100 = 10%

The amount of water = denominator X moisture %

= 5 x 0.10 = 0.5

Dry weight basis

The amount of water lost divide by the weight of the sample after drying multiply by 100.

Moisture content % (**dry weight basis**)= $\frac{\text{sample weight before drying}-\text{weight after drying}}{\text{weight after drying}}$ X 100

$= \frac{5-4.5}{4.5}$ x100 = 11.11

Amount of water = denominator X moisture %

= 4.5 x 0.111 = 0.499

Since the weight after drying is less than the weight before drying, dry weight percentage will be slightly greater than wet weight percentage. The moisture content on dry basis usually used in chemical constituents determination.

Procedure;

Moisture analysis is carried out on independently-drawn duplicate working samples, weighted to an accuracy of 1 gm. Most species are dried for one hour at 130 ºC, expect for cereals (two hours) and maize (four hours). Also, seeds containing oil are dried for 17 hours at 105 ºC.

Low = low temperature (101-105ºC) for 17± 1h.

High = high temperature (130-133ºC) for 1h ± 3 min. 2h ± 6min, or 4h ±12min.

Each empty container is weighed with its cover. The submitted sample is mixed thoroughly with a small spoon and two portions of 5.0 g each are weighed along with the containers. The seed should be distributed evenly over the bottom of the container. After weighing the containers are placed on top of their covers on the tray in the pre-heated oven. After drying the containers are closed with their covers allowed to cool for 30 minutes in desiccators and weighed again.
The moisture content (M) is calculated to one decimal place using the formula:

$$\mathbf{M = M_2 - M_3 / M_2 - M_1 \times 100}$$

M_1 = weight of empty container with cover
M_2 = weight of container with cover and seeds before drying
M_3 = weight of container with cover and seeds after drying

The result of the duplicate determinations should not differ by more than 0.2 %; otherwise, the analysis should be repeated in duplicate.

Physical Purity Test and Determination of Other Species

Purity analysis is the composition by weight of pure seed, other seed, and inert matter in a sample. Good seed should not have a high percentage of chaff, straw, and sand and weed seeds. In practice, however, it is impossible for cleaning achiness to completely remove all these admixtures. Purity analysis determines exactly how much of the impurities remain, even if the percentage is very low.

Many countries, as well as the European Economic Community, have seed regulation to protect the buyer which prescribes the minimum quality standards for seed lots. Since germination tests are based on pure seed components, it can readily be seen that purity analysis and germination tests complement each other. Thus the

actual planting value (agricultural value) of seed can be determined only when the purity analysis and germination tests are considered together.

Objectives of the purity test

The purity test has **two** objectives.

The first is to determine the composition by weight of the sample being tested and by inference the composition of the seed lot. In other words, the composition of the sample is expressed as weight percentages. If a representative sample is used, the test results apply to the lot as well.

The second objective is to determine the identity of the various species of seeds and inert matter particles in the sample. Thus, all seeds must be identified be scientific name, using the ISTA list of stabilized plant names.

Purity affects the number of pure seeds per kilogram, and therefore, the price and quantity requirements for sowing

Purity is affected by the level of seed extraction.

Purity is needed before seed sales; before seed storage; during seed processing.

Definitions of the three purity test components:

The purity test procedure separates the samples particles into three groups. Since 1976 the international certificate has listed only three components; "other crop seed" and "weed seed" are now combined into one fraction called "other seeds", since a species considered a weed in one country may be used as a crop in another. The ISTA rules define the three components as follows:

a) Pure seed: the pure seed shall refer to the species stated by the sender, or found to predominant in the test and shall include all botanical varieties and cultivars of that species. The following structures (even if immature, undersized, shriveled, diseased or germinated, provided they can definitely be identified as of that species) shall be regarded as pure seed:

- Intact seeds (seeds in the botanical sense).
- Pieces of seeds, achenes, mericarps and caryopses, resulting from breakage, that are more than one-half their original size. However, leguminosae and cruciferae seeds with the seed coats entirely removed shall be regarded as inert matter.
- Florets and caryopses of gramineae as follows:

I – florets and one-flowered spikelets with an obvious caryopsis containing endosperm. A sterile floret.

II – free caryopsis and pieces of caryopsis resulting from breakage that are more than one-half their original size.

b) Other seed: other seeds include seeds and seed-like structures of any plant species other than that of pure seed. With respect to classification as other seeds or inert matter, the distinguishing characteristics set out for pure seed shall also be applicable to other seeds.

c) Inert matter: inert matter shall include seeds, seed-like structures and other matter as follows; pieces of broken or damaged seeds, achenes, mericarps one-half the original size or less, soil, sand, stones, stem, leaves, and pieces of bark, flowers, nematode galls, fungus bodies (ergot, sclerotic) and all other matter that is not seed.

Experimental Procedures:

a) Working sample: the purity analysis shall be made on a working sample taken from the submitted sample.

b) Separation:

- The sub-sample is spread on the working table
- Each particle is judged individually based upon external appearance (shape, size, color, gloss, surface texture).
- All other seeds and inert matter particles are removed, leaving the pure seed.
- Seed enclosed in fruits other than those cited under "pure seed" shall be separated and the detached empty fruit classed as inert matter.
- After separation each component part any species of seed and any type of other matter which requires a reported percentage shall be weighted in grams to the minimum number of decimal places necessary to calculate the percentage.

Test equipment:

1 – Optical aids
2 – Balances
3 – Weighing table
4 – Sieves
5 – Other equipment. Such as spoon, spatulas, scalpel, and shallow trays.

Weight of each component % and calculate to a percentage using the formula below:

$$= \frac{\text{weight of the component g}}{\text{Sum of weights of the components g}} \text{ X } 100$$

Seeds Test Weight

Objectives

It is a quality determination and it must be within the required limit. The weight tends to increase as moisture content decreases, so the test should be taken as quickly as possible after the grain has been delivered to the laboratory.

Test weight is the weight in kg of one litter level full volume of grain multiplied by 100 to express as kg per hectoliter, it is **bulk density**.

Seeds bulk density affected by various factors, seed arrangement within the container and spaces between seeds, seed size, shape and chemical components. As the specific gravity of oil is less than the starch or protein, therefore the test weight of oily seeds is lower than those of starchy and proteineous seeds. Low volume weights are an indication of immaturity, insect damage, drought effects, frost damage and sterility.

Seed index (thousand seed weight) are sometimes desirable as a measure of seed quality in small grains (*Triticum, Oryza, Hordeum, Sorghum* etc.).

Seed index test can be determined either on air-dry seed (absolute), or oven dry seed. It is an index of the size and plumpness of air dried seeds expressed in grams. Seed index is influenced by environments factors and cultural practices. For samples comparison, the absolute weight (dry matter basis) of the seeds must be determined according to the formula:

$$\text{Absolute weight on dry matter basis} = \frac{1000 \text{ seed weight at test X dry matter}}{100}$$

$$\text{Absolute weight} = \frac{1000 \text{ seed weight at test}(100-\text{moisture}\%)}{100}$$

Therefore seeds of 14.5% moisture content with 30 gm weight of thousand seeds and seeds of 20% moisture content with 32 gm weight of thousand seeds will have the same absolute thousand seeds weight.

Absolute weight = $\frac{30(100-14.5)}{100}$ = 25.65

Absolute weight = $\frac{32(100-20)}{100}$ = 25.60

In general, the higher the volume weight or weight per 1000 seeds, the higher is general seed quality.

Materials and Equipment

Hectolitre apparatus, electronic seed counter, counting board, vacuum counter, different types of crop seeds.

Experimental Procedures

Take two replicates of 1000 seeds of different crop and record the weight.

Take two replicates of hectoliter weight

In the case of quarter liter volume the following formula can be used to estimate hectoliter.

Test weight (kg/hl) = $\frac{\text{weight (g) of quarter litre X 4 X100}}{1000}$

Reporting of the Results and Interpretation

Samples	Kg / hectolitre	Mean	1000 seeds weight (g)	Mean
Wheat,replicate 1				
Wheat,replicate 2				
Barley, replicate 1				
Barley, replicate 2				
Sunflower, replicate 1				
Sunflower, replicate 2				

Phenol Reaction Test

The phenol color test provides a useful tool for varietal identification, which is an important aspect of a good seed production program. Since Piper (1922) showed that phenol tests could be used to identify wheat cultivars, several workers have standardized this test. The use of phenol color reaction is now one of the recommended tests for identification of wheat cultivars.

The phenol test is a very simple method for classifying wheat cultivars into different color group. Biochemical studies on phenol color reaction have shown that it involves the enzyme tyrosinase using phenol as a substrate. The seed coat is the site of biochemical reaction giving black, dark brown mars brown, brown or snuff brown and negative color. Often, however, more than one cultivar falls into a particular color group, making it difficult to identify a particular variety.

Materials and Equipment

Wheat seeds - Phenol (carbolic acid), Petri-dishes- filter paper- pipette- incubator.

Experimental procedure:

Soak two replicates of different cultivars of 100 wheat seeds separately in tap water for 16 hours at 20ºC ± 1. Remove the water from the surface of the seeds and transfer to Petri-dish lined with two layers of filter paper and moisten with about 4 ml of 1% phenol solution (take care- phenol is caustic). Incubate at 30º C for about two- four hours, the phenol colour reaction is noted, which is actually is the change in the colour of the seed coat, in wheat the entire pericarp is observed for degree of staining. Note the color reaction according to the following scale: black (+ + + +), dark brown (+ + +), brown (+ +), light brown (+) and negative (-).

Then repeat the test with **Tyrosine** (**1**%) instead of phenol and also repeat the test with the medical cream (**Hydroquinone 4**%) then note the color reaction according to the scale: black (+ + + +), dark brown (+ + +), brown (+ +), light brown (+) and negative (-).

This is basically a chemical test in which the reaction takes place in the seed coat. Some researchers suggested that the colour change in wheat was caused by a chemical reaction of nitrogen compounds in the presence of metal and oxygen. Others considered that the reactions is of enzymatic nature of the coloration, as the detection of tyrosinase enzyme (monophenolase) is the basis of the phenol reaction in wheat. While in case of negative phenol reaction a low tyrosinase activity was manifested.

Biochemical studies on phenol colour reactions have shown that the enzyme tyrosinase is involved, and that it uses phenol as a substrate. Tyrosinase oxidizes the amino acid tyrosine to dihydroxyphenylalanine, which in turn is oxidized to o-quinon.

On the other hand, reseachs reported that tyrosinase also converts tyrosine to melanin, which is responsible for colour reaction, and referred to quinon as a compound derived from benzene, which function in biological oxidation-reaction systems. In the case of barley higher phenol concentration, higher temperature and longer treatment time were required for colouring of barley seeds.

Seed Germination Test

The aim of the germination test is to furnish reliable information on the field planting value of a seed. The test results can be used to compare the quality of different seed lots. The germination test must produce results that are uniform and reproducible within and between seed testing laboratories.

Germination in a laboratory test, is the emergence and development of essential structures from the embryo of the tested seeds, it is an indication of the ability to develop into a normal plant under favorable conditions in the field. The seeds attain maximum germination and vigor at physical maturity, while still at high moisture content. The ultimate objective of germination test is to obtain information with respect to field planting value (Agricultural value) of the seed. It involves determination of seeding rate as it has been mentioned earlier.

Procedures and General principles:

The germination test should always be done on seeds from the pure seed fraction. From well-mixed pure seed 400 seeds are counted at random into replicates of 100, 50, or 25 seeds. The seeds are spaced uniformly on a moist substratum, sufficiently separated to facilitate easy seedling evaluation and to prevent the seedling from touching one another before they are counted and removed. This helps to prevent the spread of any infections.

The replicates are then placed under optimal germination conditions, usually including a treatment to break dormancy if needed. The first count is made when the

majority of seedlings have reached the developmental stage at which proper evaluation is possible. The normal seedlings are removed and counted. Rotten seeds and decayed seedling are also removed to prevent contamination and counted. The final count also records the number of hard and fresh ungerminated seeds. If some seeds start to germinate only at the end of the test, the test can be prolonged. When the results of the test replicates fall within the maximum tolerated range, the average of the normal seedlings represent the percentage germination.

Preparing the substratum

Substratum used in the germination test include paper, sand and soil depending on the laboratory's germination facilities. The substratum must be non-toxic and relatively free of molds other microorganisms and their spores. It must also provide adequate aeration and moisture for germinating seeds. To cut down on watering after planting, the relative humidity of the air around the seeds should be kept as close to saturation as possible. The pH value of the paper and sand should be 6.0 – 7.5

Water

Tap water can be used to moisten the substratum, provided it is reasonably free from acidic, alkaline, organic or other impurities. Otherwise, distilled or deionizer water should be used.

Paper

All paper substratums should be porous, but with a texture fine enough to prevent seedling roots from growing into the paper. For most seed types, the paper should not be so wet that a film of water forms around the finger when the moistened paper is

pressed. Filter paper, blotter paper, or paper towels can be used to germinate seeds. Seeds can be placed on top of one or more layers of paper or, alternatively, germinated between two layers of paper. Pleated paper seems to be a very good substratum for germinating seeds that are pelleted and those very susceptible to surplus moisture, because making it possible to regulate moisture.

Sand

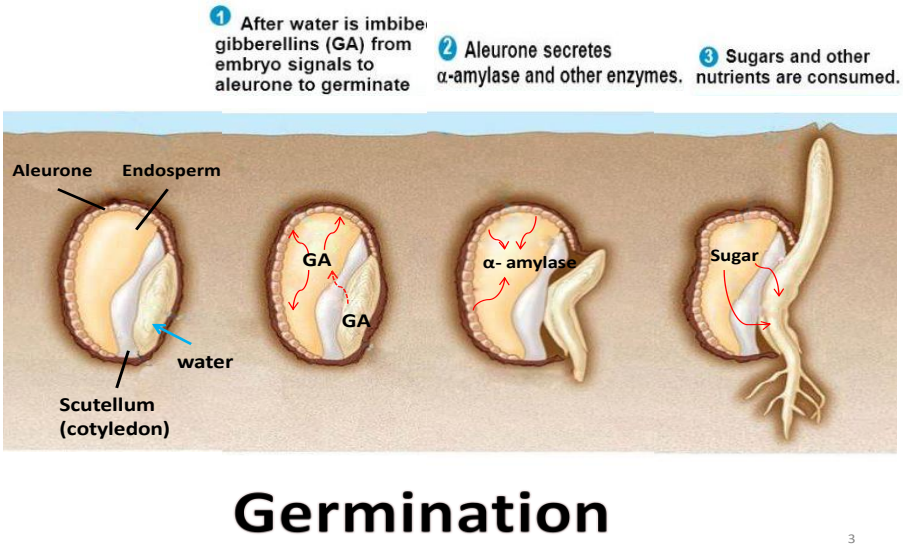
Sand is normally as a substrate for larger seeds such as cereals, peas and beans. Depending on their size, the seeds can either be planted on a layer of sand and covered with 10-20 mm of loose sand or planted atop the sand and pressed into its surface. The amount of water added to the sand depends on the seeds characteristics and size, but the sand should not be so wet that optimal aeration is prevented. The top and bottom layers of sand should be raked to enable good gas exchange.

The sand bed should then be prepared, first by filling the germination pan with a leveled layer of moist sand and then loosing the sand with forceps or a rake. The sand surplus is removed with the scraper but without pressing the sand.

Soil

Soil or an artificial compost is commonly used instead of to test samples that produce seedling with phytotoxic symptoms when germinated in sand or paper. Such seedling may become normal if the toxic substance can be absorbed by the humus complex of the soil.

The soil test is also used to confirm the evaluation of seedlings in doubtful cases, but it is not recommended for routine germination tests since soils are more difficult to standardize and therefore liable to cause greater variation between test results.



Evaluation of the germination test

The degree of correctness with which seedlings are assessed greatly influences the uniformity of test results. Seedlings can only be properly distinguished as normal or abnormal at a developmental stage in which all essential structures can be inspected.

1. Normal seedling

A normal seedling shows the capacity for continued development into a normal plant when grown in good quality soil with favorable water supply, temperature and light. Normal seedling can be completely intact or have slight defects or secondary

infections. Detailed classification of normal seedlings is given in the ISTA handbook for seedling evaluation. Some examples of seedling with slight detects are:

- Seedlings of which the primary root, hypocotyls or epicotyls shows limited damage such as discolored or necrotic spots, splits or cracks which are healed or limited in depth.

- Seedlings of Zea, all Malvaceae and Cucurbitaceae species and large-seeded leguminosae with a badly damaged primary root, but with a sufficient number of secondary roots.

- Seedling of dicotyledons of which the cotyledons or the primary leaves are damaged, but not to such a degree that more than 50% of the total area of the cotyledons or primary leaves is not functioning normally.

2. Abnormal seedling

An abnormal seedling does not show the capacity to develop into a normal plant when grown under favorable conditions in good quality soil. Detailed classification of abnormal seedlings is given in the ISTA handbook for seedling evaluation. The three major classes of abnormal are:

a) **Damaged seedling**, in which any essential structure is missing or badly damaged.

b) **Deformed or unbalanced seedlings** with an abnormality often caused by internal disturbances of a physiological/ biochemical character (e.g., chlorophyll deficiency, roots with negative geotropism and twisted, spindly or glassy seedling).

c) **Decayed seedlings** in which any essential structure is diseased or decayed from primary fungal or bacterial infection to the extent that normal development is prevented.

3. Hard seeds

Seeds that remain hard at the end of the test because the structure of the seed coat does not allow water absorption are classified as hard seeds (Leguminous and Malvaceae).

4. Fresh ungerminated seeds

Seeds that are able to imbibe water but remain firm and apparently viable, even after appropriate treatment to break dormancy, are classified as fresh ungerminated seeds.

5. Dead seeds

Ungerminated seeds that are neither fresh nor hard are classified as dead seeds. They can be removed from the substratum at any count, but they must clearly be rotten. (Empty and insect-damaged seeds are usually considered dead seeds as well).

Tetrazolium test for seed viability

Objectives

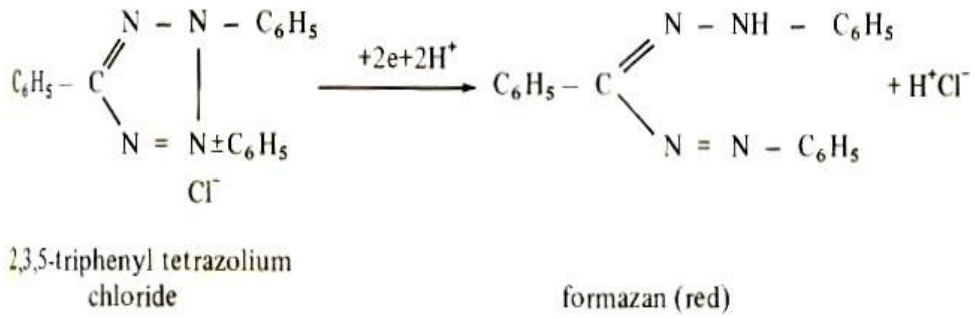
Topographical tetrazolium or TZ test is very useful for rapidly obtaining an indication of germination potential and viability of samples and is in extensive use.This test has been accepted as an official method by ISTA and AOSA.

In this biochemical test, living cells are made visible by reduction of an indicator dye. The indicator used in the TZ test is a colourless solution of a tetrazolium salt imbibed by the seed. Although a number of tetrazolium compounds can be used; 2,3,5 triphenyl tetrazolium chloride is preferred.

Within the seed tissues, it interferes with the reduction processes of living cells and accepts hydrogen from the hydrogenases. By hydrogenation of the 2,3,5-triphenyl tetrazolium chloride, a red stable and non-diffusable substance, triphehyl Formazan, is produced in living cells.

This chemical is reduced to an insoluble red coloured formazan in the presence of dehydrogenase group of enzymes. These enzymes are essential for seed respiration and hence, viability. Therefore, the live seeds or living tissues develop red colour called formazen for a period of time usually (1-2 hr). Seed viability is judged on the basis of the pattern and the intensity of red colour developed in their embryos.

The reaction is as follows:



The chemical reaction that changes the colorless tetrazolium solution into formazan.

Method

1 – Wheat and chickpea seeds should be soaked overnight in water at room temperature.

2 – The water-soaked seeds are then cut longitudinally (e.g. wheat, maize) or laterally (e.g. small-seeded grasses) to expose the embryo. Seed coats of dicots (e.g. chickpea) should be removed to facilitated the quick penetration of tetrazolium.

3 – After the desired number of seeds are prepared they should be soaked in 1.0 % solution of tetrazolium (TZ) of pH 6-7 and kept, preferably in darkness, at 30ºC for 3-4 hrs.

4 – When the color has developed the TZ solution should be drained and the seed should be rinsed two or three times with water and evaluated. During evaluation seeds should be immersed in water.

Blending in seed conditioning

Blending" is the process of mixing the seed of two cultivars prior to planting. or Blending is the mixing or intermingling of two or more different seed kinds to produce a final lot of overall uniformity.

Blending seed "isn't a new concept, but the research aspect of it is**.**

Blending seed, either within the same lot or from two or more lots, is a common practice. A single lot of seed will have areas of dissimilarity in test weight, germination, concentration of weed seed, other crop seed, or inert matter. This is especially true if the lot is made up of seed from several different fields. Blending a lot, to mix the different parts of the lot thoroughly with each other, makes it more uniform in quality.

Reliable seedsmen make it more every effort to market seed of uniformly high quality. It is often necessary to lend two or more lots to produce a final lot of the required uniform quality. A lot of seed with slightly substandard germination can be blended with a lot of usually high germination, to produce one large lot of acceptable germination. In the same way, small amount of crop seed or common weed seed that are not troublesome in the field but are difficult to remove conditioning, can be diluted to give a product which equals or exceeds the quality required.