Study Material

M.Sc. Ag. IV semester
Course: Principles & Practices of Seed Production
Chapter: 06 (Seed Testing)

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TOPICS:

A) Introduction to Seed Testing
B) International Organizations for Seed testing
C) Seed Sampling (This Topic has Already been taught in Class)
D) Physical purity Analysis
E) Germination and Germination test
F) Seed Moisture Estimation
G) Seed Dormancy
H) Seed Viability testing by TZ method

Topic:

Introduction to Seed Testing

What is seed testing: The science of see testing, that is, the science of evaluating the planting value of the seeds. Seed testing helps to assess the quality attributes of the seed lots which have to be offered for sale and minimizing the risk of planting low quality seeds.

Objectives Seed Testing

- To determine the seed quality i.e., their sustainability for planting.
- To identify seed quality problems and their probable causes.
- To determine the labelling specifications.
- To establish quality and provide a basis for price and consumer discrimination among lots in the market.
- To determine the need for drying and processing.

Historical Event

1869- The 1st Seed Testing Lathe station was established in Thrandt, Saxony, Germany by Friedrich Nobbe .
1876- Hand book of seed testing was published by Friedrich Nobbe
1871: E. Moller Holst, established a seed testing lab in Copenhagen, Denmark
1876 -1st Seed Testing station in US
1900- Europe 130 Seed Testing Stations
1924- International Seed Testing Association (ISTA)-to devlop procedures and to promote uniform application.
1939 -Association of official Seed Analysts (AOSA)
1960 -First Seed Testing Laboratory was established in India (CSTL at IARI, New Delhi)
1967 -First seed testing manual was published for uniform testing.

Topic:

INTERNATIONAL ORGANIZATIONS

The International Seed Testing Association (ISTA): was founded in 1924 during the 4th International Seed Testing Congress held in Cambridge, UK. It is an independent organisation supported by the non-profit cooperation of experienced seed scientists and analysts. Membership consists of Member Laboratories and sampling entities, Personal Members and Associate Members, from 83 countries/distinct economies. More than 130 of the Member Laboratories are accredited by ISTA and entitled to issue **ISTA Certificates**. The membership is a diverse collaboration of seed scientists and analysts from universities, research centres and seed testing laboratories around the world. ISTA works in developing standard seed testing methods facilitates the trade of quality seeds and makes a valuable contribution to food security.

Objectives of ISTA

1. To develop, adopt and publish internationally agreed standard procedures (Rules) for sampling and testing seeds.
2. To promote uniform application of standard procedures for evaluation of seeds involved in international trade.
3. To award accreditation to laboratories.

4. To actively promote research and dissemination of knowledge in seed science and technology, for the sampling, testing, storing, processing and distribution of seeds.
5. To provide international seed analysis certificates and training courses.
6. To encourage variety (cultivar) certification.
7. Participation in conferences, strengthening links to other organisations with seed interests, such as **ISF** (International Seed Federation), **OECD** (Organisation for Economic Cooperation and Development), **UPOV** (International Union for the Protection of New Varieties of Plants), **WFO** (World Farmers' Organisation) and many others.
8. Publication of the **ISTA International Rules for Seed Testing** – globally available and annually updated, harmonised, uniform seed testing methods
9. **ISTA Accreditation Programme**, including Accreditation Standard, Proficiency Testing and Auditing Programme, guaranteeing worldwide, harmonised, uniform seed testing.
10. An issue of **ISTA Certificates for seed analysis certificates** by independent ISTA-accredited laboratories.
11. Global platform for research, professional development, training, publishing and information dissemination, established to promote education and experience in all areas of seed science and technology.

ISTA Executive Committee

The Executive Committee manages the affairs of the Association. According to Article 15 (a) of the Articles of ISTA, the Executive Committee shall consist of the President, Vice-President and Immediate Past President, together with eight members-at-large.

ISTA Secretariat:

Manages the finances and administration of the Association. This is led by the Secretary General and supported by eleven members of staff.

The headquarters of the Association is located in Zurich, Switzerland.

Official language- English, French and German

ISTA Technical Committees:

The Technical Committees perform comparative studies and surveys in different research fields. They develop and enhance the ISTA 'International Rules for Seed Testing' and ISTA Handbooks on seed methods including sampling, testing and processing seeds, and are responsible for the organisation of Symposia, Seminars and Workshops. ISTA Technical

Committees regularly hold workshops which provide a platform for the exchange of information, experience and ideas.

1. Advanced Technologies Committee
2. Bulking and Sampling Committee
3. Editorial Board of Seed Science and Technology
4. Flower Seed Testing Committee
5. Forest Tree and Shrub Seed Committee
6. Germination Committee
7. GMO Committee
8. Moisture Committee
9. Nomenclature Committee
10. Proficiency Test Committee
11. Purity Committee
12. Rules Committee
13. Seed Health Committee
14. Seed Science Advisory Group
15. Statistics Committee
16. Seed Storage Committee
17. Tetrazolium Committee
18. Variety Committee
19. Vigour Committee
20. Wild Species Working Group

ISTA certificates

Orange seed lot certificate: Sampling & testing is carried out in same country , where the seed lot is located. ISTA accredited seed testing laboratory takes responsibility of the sampling and sealing of the seed lot as well as the testing of the seed. In issuing an orange certificate the accredited laboratory takes responsibility that the results are not only representative of the sample but also the seed lot from which the sample was drawn.

The green Seed lot certificate: it is same as orange, except the testing is carried out other member country authorised by ISTA. This is for the situation where the seed lot is in one country and the testing needs to be carried out in another country. The accredited seed testing laboratory in the country where the seed lot is situated arranges for the seed lot to be sealed and sampled. Subsequently, instead of testing the sample, the laboratory forwards it to the accredited laboratory in the other country where the sample is to be tested with details of the seed lot and its sealing and sampling.

The blue Seed sample certificate: when sampling is done unofficially. Seed testing station is not certain whether the sample represent the seed lot. Certificate refers only the quality of sample received not for the lot. It covers the situation where only information about a sample is asked or the accredited seed testing laboratory has no jurisdiction over the drawing of the sample and is therefore not in a position to verify that it has been drawn correctly and is representative of the seed lot. The accredited seed testing laboratory in issuing a blue certificate only takes responsibility for the seed test carried out on the sample submitted for testing.

Publications for International Seed Testing Association:

1. International Rules for Seed Testing
   Currently available:
   Volume 2015, Number 1, 1 January 2015 - Volume 2020, Number 1, January 2020
2. Seed Science and Technology(Quarterly)
   Currently available:
   Volume 31, Number 1, April 2003 - Volume 48, Number 1, April 2020
3. Seedling Evaluation Handbook
   Currently available:
   Volume 1, Number 1, 1 January 2018 - Volume 1, Number 1, 1 January 2018

Association of Official Seed Analysts (AOSA)

The Association of Official Seed Analysts (AOSA) is an organization of member laboratories. Members include official state, federal, and university seed laboratories across the United States and Canada.

Membership is extended to allied laboratories (those of government agencies and institutions outside the associate members (individuals not assigned to a member laboratory but

contribute in a supportive role), and honorary members (those who have distinguished themselves in contributions to the Association and /or industry)

1908 – Formed as AOSA of North America
1939- The phrase North America was removed, the AOSA

Objectives:

1. An attempt to seek uniformity and accuracy in methods, results, and reports.
2. To make effort efforts to develop, adopt and publish uniform rules for seed testing
3. To ensure that testing procedures that are standardized between analysts and between laboratories.

Publications of AOSA: Journal of Seed technology and News letter

The Society of Commercial Seed Technologists (SCST)

SCST is an organization of professional seed technologists dedicated to research, proficiency and improvement in seed testing from private and company laboratories across the United States.

The Society of Commercial Seed Technologists is a organization comprised of commercial, independent and government seed technologists. Formed in 1922, the SCST functioned as a liaison between the Association of Official Seed Analysts (AOSA) and the American Seed Trade Association (ASTA). The SCST has developed over the years into a progressive organization that trains and provides accreditation of technologists, researches and develops rule changes, publishes training and education materials, and serves as an important resource to the seed industry.

METHODS /PROCEDURES OF SEED TESTING

1 .Seed sampling
2. Physical purity
3. Germination testing
4. Seed moisture testing
5. Seed viability testing
6. Seed health testing
7. Seed Vigour Testing
8. Determination of Genuineness of cultivars

Topic:

Seed sampling (This topic has already been taught in class)

Seed sampling is aimed at obtaining a sample of the required size and consisting of the same components as the whole lot of seeds. The quantity of seed tested in the laboratory is small, compared with the size of the seed lot, which it is intended to represent. No matter how accurately the laboratory work is done, the results can only show the quality of the sample submitted for analysis. Consequently, every effort must be made to ensure that the sample sent to the seed-testing laboratory accurately represents the seed lot in question. Primary Sample: a fraction of seeds taken randomly from seed lot different places.

A sample is obtained from the seed lot by taking small portions at random from different position of the lot and combining them.

Seed lot: It is specified quantity of seed, physically identifiable, in respect of which a seed test certificate can be issued.

Types of Seed Samples

There are three types of seed samples received by a seed-testing laboratory.

(1) Service sample (2) Certification sample (3) Official samples

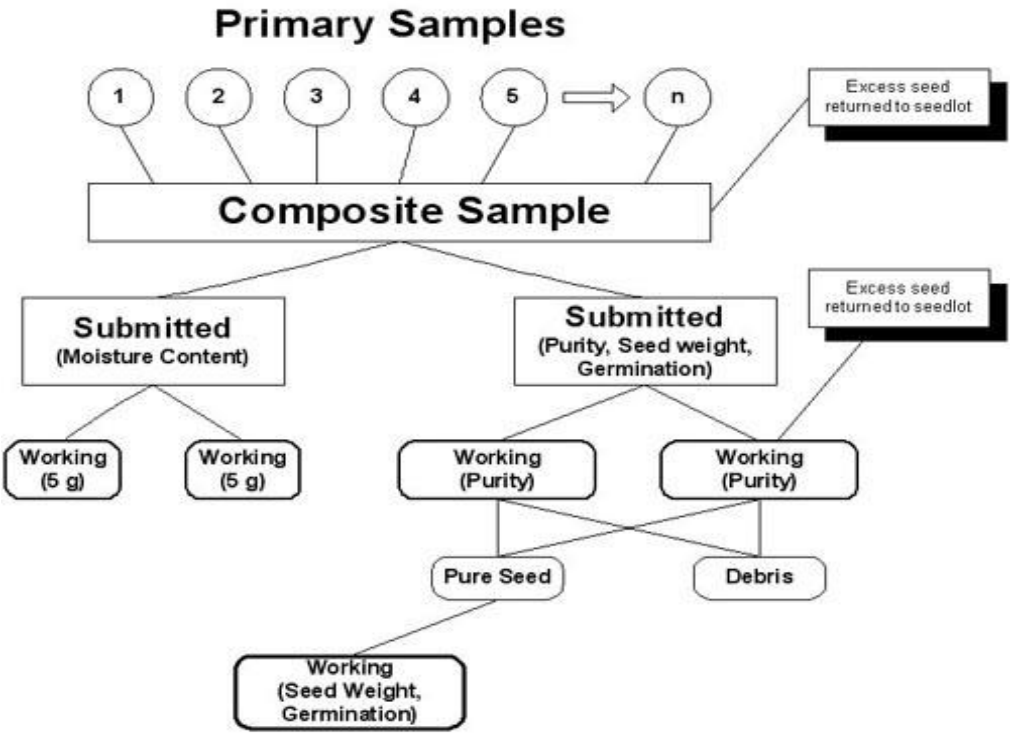
1. **Service Samples** means a sample submitted to the central seed testing laboratory or to a state laboratory for testing the results to be used as information for seeding, selling or labelling purposes. Any private individual i.e Farmer, seed dealer or any individual /agency can send sample to know the quality status of seed lot. The main purpose of analysing service sample is to provide results for sowing or labelling purpose.

2. **Certification Sample:** Certification sample means a sample of seed drawn by a certification agency or by a duly authorized representative of a certification agency established under section 8 or recognized under section 18 of the Seed Act 1966. The main purpose of such sample is to determine that sample in question confirms the prescribed quality standards for the purpose of seed certification or not.

3. **Official Samples Or Seed Law enforcement sample:** : Official sample means a sample of seed drawn by recognised official of seed law enforcement agency (Seed inspector) to ascertain that the seeds meet minimum limit of specified quality. All possible efforts are made to start testing a sample on the day of receipt. However under unavoidable circumstances the sample shall be stored in dry cool, ventilated room so that changes in the quality of seed are minimized

The purpose of such sample is to know the quality status of a lot for the purpose of seed legislation.

Types of sample drawn at various stages



- **Primary sample**: is the small portion taken from a lot at particular stage.
- **Composite Sample**: Formed by combining & proper mixing of all primary sample. Or pooled sample of all primary samples.
- **Submitted Sample**: sample which is submitted to seed testing laboratory and obtained from composite sample.
- **Working Sample**: Working Sample is a sub sample taken from Submitted Sample in the laboratory on which one of the quality tests is made.

(All procedures of sampling already been covered) pl refer Text book: RL Agrawal for revision purpose).

Topic:

Physical purity Analysis

What is a Purity Test?

A purity test is conducted on approximately on 2,500 seeds which are broken down into four components: Pure seed, Other crop seed, Weed seed, and Inert matter. The components are then weighed from which percentages are calculated. The percentage is based on the weight of each component, not the number of seeds.

Need for physical purity analysis

a) Seed Certification or Seed Law Enforcement Agencies to judge that the seed lot conforms to the prescribed standards.

b) Seed processing plants for using right kind of processing equipment.

c) Physical purity analysis is a pre-requisite for germination test because 'pure seed' component is used for germination testing.

Objective: The primary objective of physical purity analysis is to determine-

i) The percentage composition by weight of the sample being tested and by inference the composition of seed lot

ii) The identity of various species of seeds and inert particles constituting the sample.

Requirements for Purity analysis

Purity table or working board, Seed divider, Forceps, Spatula, Brush Aluminum purity dish, Magnifier (5 to 7x), Analytical balance, Hand screen (with ISI specifications), Top loading balance (as mentioned above), Stereoscopic binocular microscope, Seed blower, Table lamp, Watch glass

Components of Seed physical purity Analysis

- **Pure seed**: Seeds of kind/species stated by sander or found to predominant in the test.
- **Other crop seeds**: seeds of plants which are as crops, other than main crop.
- **Inert matter**: includes seed units and all other matter and structures not defined as pure seed, other crop seed or weed seed.

- **Weed seeds**: Seeds of a weed species which are recognized as weeds by law/general usage.

Method/ Procedure of Purity Testing

1. Obtaining Working sample

Since the size of the working sample is minute as compared with the size of the seed lot to which it represent, it is therefore, very essential that the working sample should be obtained in accordance with the procedures. The working sample shall be either a weight estimated to contain at least 2,500 seed units or not less than weight indicated.
Boerner or soil type seed divider should be used to homogenize the submitted sample before reducing it to the size of working sample. The following guidelines need to be followed-

i. Check the cleanliness of the divider and the container.
ii. Pour the entire contents of the submitted sample into the hopper of the divider.
iii. Allow the content of the submitted sample to pass through the main body of the divider. In case of 'Soil type' seed divider this can be accomplished by tilting the hopper over the body of the divider while in case of 'Boerner' divider, by opening the gate-valve situated at the base of the hopper.
iv. Recombine the contents of both sample receiving pans and again pass it through the divider.
v. Repeat this process twice in order to homogenize the submitted sample.
vi. Divide the submitted sample.
vii. Set aside the contents of one container.
viii. Divide the contents of the other container subsequently till the weight of working sample is obtained.

2. Separation

i. Clean the work board, sample and purity dishes before starting the separation
ii. Examine the working sample to determine the use of particular aid such as blower or sieves for making separation.
iii. After preliminary separation with the help of sieves or blower, place and spread the retained or heavier portion on the purity work board.

iv. With the help of spatula or forcep, draw working sample into thin line and examine each particle individually. The criteria used being the external appearance (shape, size, color, gloss, surface texture) and/on appearance in transmitter light.

v. Separate out impurities such as other crop seeds, weed seeds and inert matter and place the impurities separately in purity dishes, leaving only the pure seed on the purity board.

vi. Seed enclosed in fruits other than those indicated in pure seed should be separated and the detached empty fruit/appendages classed as inert matter.

vii. Collect the pure seed in the sample pan.

viii. Put the lighter portion of the work board and examine under magnification for further separating into the requisite classes (other crop seed, weed seed and inert matter).

ix. After separation, identify the other crop seed, weed seed and record their names on the analysis card. The kind of inert matter present in the sample should also be identified and recorded.

x. Weight each component, pure seed, other crop seed, weed seed and inert matter in grams to the number of decimal places shown below:

Wt. of working Sample (g)	**No of decimal place required**	**Example**
Less than 1	4	.9025
1 to 9.990	3	9.025
10 to 99.99	2	90.25
100 to 999.99	1	902.5
1000 or more	0	1025

xi. Calculate the percentage by weight of each component to one decimal place only, basing the percentage on the sum of the weight of all the four components. If any component is ***less than 0.05%*** record it as *'**Trace**'. Component of 0.05% to 0.1% are reported as 0',1%.*

During purity analysis, each ‘pure’ seed fraction from the working sample is separated from the inert matter and other seeds. %age Weigh the ‘pure’ seed fraction should be express as the purity percentage . Weight of pure seed over the total weight of the working sample is calculated as shown below-

A purity percentage is calculated as:

Purity (%) = Weight of pure seeds (g) x 100 /Total weight of working sample (g)

Reporting of Results

- Weight by percentage Single decimal place
- All components should add to 100 %
- Less than 0.05% reported as ‘trace”
- Any component is found to be nil- reported as -0.0-
- The components scientific names should be mentioned

Topic:

E) Germination

Definitions :

Seed germination is a physiological process, its consecutive numbers of step which causes quiescent seed with low moisture content to show a rise in its general metabolic activity and initiate formation of seedling from embryo **Or**

Seed Germination is the process by which a seed can reproduce an organism / healthy Seedling that lead to healthy plant. **Or**

Seed germination is the resumption of active growth of the embryo that results in the rupture of the seed coat and the emergence of the young plant under favourable conditions.

Or

Seed germination is a process by which the embryo in the seed becomes activated and begins to grow into a new seedling.

Types of germination:

Hypogeal germination: Hypogeal germination implies that the cotyledons stay below the ground. The epicotyle (part of the stem above the cotyledon) grows, while the hypocotyle (part of the stem below the cotyledon) remains the same in length. In this way, the epicotyl pushes the plumule above the ground. Normally, the cotyledon is fleshy, and contains many nutrients that are used for germination. No photosynthesis takes place within the cotyledon.

Plants that show hypogeal germination need relatively little in the way of external nutrients to grow; therefore they are more frequent on nutrient-poor soils. The plants also need less

sunlight, so they can be found more often in the middle of forests, where there is much competition to reach the sunlight.

Plants that show hypogeal germination grow relatively slowly, especially in the first phase. After the slower first phase, the plant develops faster than plants that show epigeal germination.

The cotyledons or storage organs do not emerges above the soil surface; only plumule emerges above the ground. Ex: Most of the monocots and pea**,** Green peas, Horse Gram, Green gram, Maize, Coconut, Mango.

Epigeal Germination: is a botanical term indicating that the germination of a plant takes place above the ground. Epigeal germination implies that the cotyledons are pushed above ground. The hypocotyl elongates while the epicotyl remains the same in length

Normally, the cotyledon itself contains very little nutrients in plants that show this kind of germination. Instead, the first leaflets are already folded up inside it, and photosynthesis starts to take place in it rather quickly.

Plants that show epigeal germination need external nutrients rather quickly in order to develop, so they are more frequent on nutrient-rich soils. The plants also need relatively much sunlight for photosynthesis to take place. Plants that show epigeal germination grow relatively fast, especially in the first phase when the leaflets unfold.

The cotyledon or storage organs emerge above the soil surface. Ex: Most of the dicots i.e Common Beans, Castor, Sunflower, Pumpkin, Watermelon, Cucumber, Gourds, Lilies

Viviparous germination: Viviparous germination is a special type of germination found in plant which are salt-loving and are thus halophytes. In viviparous germination the seeds get germinated being attached to the parent plant. The embryo grows out of the seed and then out of the fruit and projects out as a seedling with roots and hypocotyl. Viviparous plants produce seeds that germinate before they get detach from the parent plant. This phenomenon is commonly observed in mangroves (for example - Rhizophora species).

Steps occurring during seed germination

(1) Imbibition

(2) Respiration / Metabolic activities

(3) Initiation of embryo growth

(4) Rupture of seed coat and emergence of seedling

(5) Seedling establishment

(1) Imbibition: *Rapid water uptake*

The first step in the seed germination is imbibition i.e. absorption of water by the dry seed. Imbibition results in swelling of the seed as the cellular constituents get rehydrated. The swelling takes place with a great force. It ruptures the seed coats and enables the radicle to come out in the form of primary root.

(2) ***Active Metabolism/ Enzymatic activity****: Major metabolic events begin*

Imbibition of water causes the resumption of metabolic activity in the rehydrated seed. Initially their respiration may be anaerobic (due to the energy provided by glycolysis) but it soon becomes aerobic as during germination the cells of the embryo resume metabolic activity and undergo division and expansion. Stored starch, protein or fats need to be digested. These cellular conversions take place by making use of energy provided by aerobic respiration. Oxygen begins entering the seed. The insoluble food is rendered soluble and complex food is made simple. These simpler food solutions, comprising of sugars and amino acids thus formed, are diluted by water and passed towards the growing epicotyl, hypocotyl, radicle and plumule through the cotyledon.

(3) Initiation of embryo growth

During germination the cells of the embryo resume metabolic activity and undergo division and expansion. Stored starch, protein or fats need to be digested. These cellular conversions take place by making use of energy provided by aerobic respiration. This Synthesis of material is reflected in increase in size of root and shoot axis (Epicotyle, mesocotyle and hypocotyle and radical)

(4) Rupture of seed coat and emergence of seedling:

After the emerging of the radicle and the plumule, the seed activates its internal physiology and starts to respire and produce proteins and metabolize the stored of food. This is a lag phase of the seed germination. Due to increase volume of seed, seed coat get ruptured and plumule and radical are ready to come out.

(5) Seedling establishment:

This is a final stage of seed germination. In this stage, the cell of the seeds are elongated and divided, which brings out the root and radicle out of the seed and cotyledons are expanded which, are the true leaves of the new plant.

Factors affecting seed germination:

1. Water: Water is a basic requirement for germination. It is essential for enzyme activation, breakdown, translocation and use of reserve storage materials.

2. Oxygen: atmospheric air is composed of 79.9 % Nitrogen, 20% oxygen and 0.03 % carbon dioxide. Oxygen is required for germination of most of species. If CO_2 concentration is higher than 0.03 % it retards germination. Respiration increases sharply during seed germination. Since respiration is essentially an oxidative process, an adequate supply of oxygen is a must.

3. Temperature: Seed germination is a complex process involving many individual reactions and phases, each of which is affected by temperature. The effect on germination can be expressed in terms of cordial temperature i.e., minimum, optimum and maximum temperature. The optimum temperature for most of the seeds is between 15 to 30 0_C. maximum temperature is between 30 to 40 0_C. Some species will germinate even at freezing point also ex. Alpine

4. Light: Some species required light for seed germination. Both light intensity (lux) and light quality (colour and wavelength) influence seed germination

5. Soil factor: Soil structure, soil texture and soil temperature influences on seed germination.

GERMINATION TESTING

It is the emergence and development from the seed embryo of those essential structures which for the kind of seeds being tested, indicate the ability to develop into a normal plant under favourable conditions in the soil

Principles and Objectives of the germination test-

Germination tests shall be conducted with a pure seed fraction. A minimum of 400 seeds are required in four replicates of 100 seeds each or 8 replicates of 50 seeds each or 16 replicates of 25 seeds each depending on the size of seed and size of containers of substrate.

The test is conducted under favourable conditions of moisture, temperature, suitable substratum and light if necessary. No pre-treatment to the seed is given except for those recommended by ISTA.

The ultimate aim of testing the germination in seed testing laboratory is to obtain information about the planting value of the seed sample and by inference the quality of the seed lot. In addition, the laboratory germination results are also required for comparing the performance potential or superiority of the different seed lots. In general, the farmers, seeds men and public agencies use the germination results for the following purposes:

1. Sowing purposes, with a view to decide the seed rate to achieve desired field establishment.
2. Labelling purposes.
3. Seed certification purposes.
4. Seed Act and Law Enforcement purposes.

Material Required

Substratum

The substratum serves as moisture reservoir and provides a surface or medium for which the seeds can germinate and the seedlings grow. The commonly used substratums are sand, germination paper and soil.

1. **Sand substratum**

Size of sand particle

Sand particles should not be too large or too small. The sand particles should pass through 0.80 mm sieve and retained by 0.05mm sieve.

Toxicity

Sand should not have any toxic material or any pathogen. If there is presence of any pathogen found then the sand should be sterilized in an autoclave.

Germination tray

When we use the sand, germination trays are used to carry out the test. The normal size of the tray is 22.5 x 22.5 x 4

Method of seed placement in sand substratum

Seed in sand (S)

Seeds are planted in a uniform layer of moist sand and then covered to a depth of 1 to 2 cm with sand.cm. The tray may either zinc or stainless steel.

Top of sand (TS)

Seeds are pressed in to the surface of the sand.

Spacing

We must give equal spacing on all sides to facilitate normal growth of seedling and to avoid entangling of seed and spread of disease. Spacing should be 1-5 times the width or diameter of the seed.

Water

The amount of water to be added to the sand will depend on size of the seed. For cereals, except maize, the sand can be moistened to 50% of its water holding capacity. For large seeded legumes and maize sand is moistened to 60% water holding capacity.

2. **Paper substratum**

Most widely used paper substrates are filter paper, blotter or towel (kraft paper). It should have capillary movement of water, at vertical direction (30 mm rise / min.). It should be free from toxic substances and free from fungi or bacteria. It should hold sufficient moisture during the period of test. The texture should be such that the roots of germinating seedlings will grow on and not into the paper. Seed Germination paper is a speciality paper used as a substratum in the seed germination test. Seed germination paper ensures optimal moisture content for the most diverse types of seeds and germination forms. This paper features excellent wet strength and its special structure prevents the seed roots from growing through. Besides, the properties mentioned above, it also has high absorbent capacity and comes in standard specification of 5-7 pH range with good bursting strength.

Method of seed placement using paper substratum

Top of paper (TP)

Seeds are placed on one or more layers of moist filter paper or blotter paper in petriplates. These petriplates are covered with lid and placed inside the germination cabinet. This is suitable for those seeds which require light.

Between paper (BP)

The seeds are germinated between two layers of towel paper. The seeds are placed between two layers of paper and rolled in towels. The rolled towels are placed in the germinator in an upright position.

GERMINATION APPARATUS

Germination cabinet / Germination room/ Room germinator

These are the appropriate size of room/ chamber where temperature, relative humidity and can be regulated as per requirement of germinating seeds.

Seed counting board

This is used for accurate counting and spacing of seeds. This consists of 2 plates. The basal one is stationary and top one is movable. Both top and basal plates are having uniform number of holes *viz*., 50/100, when the plates are in different position.

After taking the sample, the top plate is pulled in such a way that the holes are in one line so that the fixed number of seeds falls on the substratum.

Vacuum seed counter

Consists of a head, pipe and wall. There are plates of 50 or 100 holes which can be fitted to the head. When vacuum is created the plate absorbs seeds and once the vacuum is released the seeds fall on the surface / container.

Impression board

Made of plastic / wood with 50 or 100 holes / pins. Here the knobs are arranged in equal length and space. By giving impression on the sand it makes uniform depth and spacing for seed.

DURATION OF TESTING

The duration of the test is determined by the time prescribed for the, final count or during the test, if it is required to break dormancy, is not included in the test period. If at

the end of the prescribed test period some seeds have just started to germinate, the test may be extended for an additional period up to 7 days. The time for the, first count is approximate on 3rd Or 4^{th} day, if the final count is to be done on 7^{th} or 8^{th} day. Seeds that are obviously dead and decayed, and may, therefore, be a source of contamination for healthy seedlings, should be removed at each count and the number recorded. First and second counts are usually taken in case of Top of Paper (TP) and Between Paper (BP) media. At the first and subsequent counts only normal and dead seeds (which are source of infection) removed and recorded.

ROUTINE LAB. GERMINATION METHODS:

Between Paper (BP)/ Roll Towel Test:

1. Soak the towel paper in try filled with water.
2. Wash the paper with running water.
3. Remove the excess from towel paper water.
4. Place two layers of wet paper towelling as substratum.
5. Check Test number provided on the Analysis Card sample and label tally each other.
6. Record the test number, crop and date of putting on the wax paper or tag.
7. Arrange seeds spaced properly.
8. Place one layer of wet towel paper over the seed.
9. Turn up two inches of the bottom edge.
10. Roll firmly from left to right and secure with rubber band in the center.
11. Place the prepared roll towel in roll towel stand or baskets.
12. Transfer the basket or roll towel stand in the germinator maintained at the desired Temperature.

Top of Paper (TP) Method:

1. Paper of known quality such as 'Sunlit' or 'Whatman' filter paper should be used.
3. The paper should be cut in the form of circles/squares or rectangles according to the Size and shape of petridish/container.
4. Put 2-3 layer of filter paper in the petridish /germination box having airtight lids.
5. Put enough water to moisten the filter paper but there should not be excess water.
7. Record the details of test like test number and date of test on the lid of the container
8. Space the counted seeds on the moist blotter/filter paper.

9. Cover the lid

10. Transfer the test in the germinator maintained at the desired temperature.

EVALUATION OF GERMINATION TEST

The germination test is evaluated as-

ISTA classified the seedlings into different categories based on the development of essential structures.

Normal Seedlings: To achieve uniformity in evaluating normal seedlings, they must conform to one of the following definitions:

a. Intact Seedlings: Seedlings that possess all the following essential structures , well developed, complete in proportion and healthy. Intact seedlings are-

- A well-developed root system including a primary root except for those plants (e.g. ceftt 1 in species of Gramineae) normally producing seminal roots of which there still are at least two.
- A well-developed and intact hypocotyl without damage to the conducting tissues.
- An intact plumule with a well developed green leaf, within or emerging through the coleoptiles, or an intact epicotyl with a normal plumular bud.
- One cotyledon for seedlings of monocotyledons and two cotyledons and seedlings of dicotyledons.

b. Seedling with the slight defects: provided they show vigorous and balanced development of the other essential structures:

- Seedlings with a damaged primary root but with several secondary roots of sufficient length and vigour to support the seedlings in soil.
- Seedlings with superficial damage or decay to the hypocotyl, epicotyl or cotyledons, which is limited in area and does not affect the conducting tissues.
- Seedlings of dicotyledons with only one cotyledon.
- Seedlings of tree species having epigeal germination when the radicle is four times the length of the seed provided all structures that have developed appear normal.

c. Seedlings with secondary infections

- Seedlings which are decayed by a pathogen with clear evidence that the parent seed is not the source of infection.

Abnormal Seedlings: Abnormal seedlings are those, which do not show the capacity for continued development into normal plants when grown in good quality soil and under favourable conditions of water supply, temperature and light.

Seedlings with the following defects shall be classed as abnormal:

a. **Damaged seedlings:**

- Seedlings with any one of the essential structures missing or badly damaged so that the balanced growth is not expected.
- Seedlings with no cotyledons, with splits, cracks and lesions or essential structures and without primary root. seedlings with no cotyledons; seedlings with constrictions, splits, cracks or lesions which affect the conducting tissues of epicotyl, hypocotyl or root; seedlings without a primary root of those species

b. **Deformed seedlings**: Seedlings with weak or unbalanced development of the essential structures such as spirally twisted or stunted plumules, hypocotyls or epicotyls; swollen shoots and stunted roots; split plumules or coleoptiles without a green leaf; watery and glassy seedlings, or without further development after emergence of the cotyledon.

c. **Decayed seedlings**: Seedlings with any one of the essential structures showing diseased or decayed symptoms as a result of primary infection from the seed which prevents the development of the seedlings

Special categories of abnormal seedlings

a. **Multigerm Seed unit**: Seed unit which are capable of producing more than one seedlings.

b. **Un-germinated seeds**: Seeds which have not germinated by the end of the test period. These are further classified as follows:

Hard seeds: Seeds which remain hard at the end of the test period because they have not absorbed water.

Fresh un-germinated seeds: Seeds which are neither hard nor have germinated but remain clean and firm and apparently viable at the end of the test period.

Dead seeds: Seeds which at the end of the test period are neither hard nor fresh and not have produced any part of a seedling. They are collapsed and milky paste comes out when pressed at the end of the test

Retesting

If the results of a test are considered unsatisfactory it will not be reported and a second test will be made by the same method or by alternative method under the following circumstances.

1. Replicates performance is out of tolerance
2. Results being inaccurate due to wrong evaluating of seedlings or counting or errors in test conditions
3. Dormancy persistence or phyto-toxicity or spread of fungi or bacteria. The average of the two test shall be reported.

Use of tolerances

The result of a germination test can be relied upon only if the difference between the highest and the lowest replicates is within accepted tolerances.

To decide if two test results of the same sample are compatible again the tolerance table is used.

Calculation and Reporting results

The result of the germination test is calculated as the average of 4x100 seed replicates. It is expressed as percentage by number of normal seedlings. The percentage is calculated to the nearest whole number. The percentage of abnormal seedlings, hard, fresh and dead seeds is calculated in the same way. These should be entered on the analysis of certificate under appropriate space. If the result is 'nil' for any of these categories it shall be reported as **‘0,.** The sum total %age of all the category of seeds (normal, abnormal, dead, hard, fresh un germinated) should be equal to 100.

$$\text{Germination (\%)} = \frac{\text{Number normal seedlings}}{\text{No. Of Seed placed for test}} \times 100$$

Topic:

SEED MOISTURE ESTIMATION

The main purpose of moisture determination of seeds is to prevent loss of seed viability till it is planted for commercial purposes.

Definition

The moisture content of a seed sample is the loss in weight when it is dried. It is expressed as a percentage of the weight of the original sample. It is one of the most important factors in the maintenance of seed quality.

Method of moisture determination

a. Moisture meters

Moisture meters estimate seed moisture quickly but the estimation is not as precise as by the air oven method.

- Electronic moisture meter
- Universal moisture meter
- Infrared moisture balance

b. Hot Air Oven Method

- Low constant temperature method (103 degree C for 17 hours)
- High constant temperature method (130 degree C for 2-3 hours)

Determination of moisture content by hot air oven method

In this method, seed moisture is removed by drying the seed sample at a specified temperature for a specified duration.

i. Weight of the submitted sample

100 g for species that have to be ground. 50 g for all other species. The sample should be submitted in polythene bag of 700 gauge (moisture proof container)

ii. Materials required

Grinding mill

This is used for seed requiring grinding. It should not cause heating of the ground material. Air currents that might cause loss of moisture must be reduced to a minimum. The fineness of grinding should be adjustable.

Container

Container of glass or non-corrosive metal (e.g.) stainless steel should be used.

Hot Air Oven

A good quality electric air oven with a thermostatic electronic temperature control for maintaining temperature with Desiccator, Analytical balance, Sieves. A set of wire mesh sieves with meshes of 0.5 mm, 1.0 mm and 4.0 mm. within ±1°C is required.

iii. Grinding

For some seeds (e.g. Cereals and Cotton) fine grinding is essential before the moisture content is determined. In such cases, at least 50% of the ground material should pass through a wire sieve with meshes of 0.5 mm and not more than 10% remain on a wire sieve with a mesh of 1.0 mm. For leguminous seeds, coarse grinding is recommended; at least 50% of the ground material shall pass through a wire sieve with meshes of 4.0 mm.

iv. Pre drying

If the species is one for which grinding is necessary and the moisture content is more than 17%. (or 10% in the case of soybean and 13% in rice) pre drying before grinding is necessary. For this purpose, two 50 g portions are weighed and placed on open trays at 130 °C for 5-10 min. If seed moisture content is about 25% or more it should be pre-dried at 70 ° C for 2-5 hours, depending on the initial water content. The pre dried seeds should be kept in a closed desiccator for cooling. Then each of the duplicate quantities is weighed separately and about 20 g is ground. The ground material is then subjected to moisture testing using a hot air-oven as described below.

v. Moisture estimation

Moisture testing is always conducted in duplicate on two independently drawn 5-10 g working samples, weighed with an accuracy of 1 mg. Most species are dried for 1 hr at 130 °C, cereals for 2 hours (130°C) and maize for 4 hours (130°C). Seeds containing high percentage of oil should be dried at 103°C for 17 hours.

vi. Procedure/steps for conducting moisture test

- Weighed the empty container along with its cover/lid
- The submitted sample should be mixed thoroughly and two small portions or seed sample are to be drawn and it should be ground as per the requirements.
- Then fill the container with4- 5 grams of ground sample and weigh it.

- After weighing, remove the cover or lid of the container and the open container should be kept in the oven which has already been heated to the prescribed drying temperature.
- At the end of the drying period, container should be closed with its cover or lid. The container should be transferred into a Desiccator. The Desiccator should be closed and the sample should be allowed to cool for 30 minutes.
- The sample should be weighed again and the moisture content may be calculated to one decimal place by the following formula-

$$m = \frac{m_2 - m_3}{m_2 - m_1} \times 100$$

Where, m = Seed moisture content (in %age)

m_1 = Weight of the empty container with its cover

m_2= Weight of the container with its cover and seeds before drying

m_3 = Weight of the container with its cover and seeds after drying

The duplicate result of the determination may not differ by more than 0.2% otherwise the analysis should be repeated.

If sample is pre dried, the moisture content is calculated from the results obtained in the first (pre-drying) and second stages of the procedure. If S_1 is the moisture lost in the first stage and S_2 is the moisture lost in the second stage, each calculated as above and expressed as a percentage, the original moisture content of the sample is calculated as below.

$$m = S_1 + S_2 \; (-) \; \frac{S_1 \times S_2}{100}$$

m= moisture content (in %age)

S_1 =Moisture percentage lost in pre-drying stage (First satge)

S_2= Moisture percentage lost in drying stage (second stage)

Topic:

DORMANCY

It is common observations that seeds of many plants species do not germinate when freshly harvested even under favourable conditions. They need a period of rest/ storage before they become capable of germination.

Inability of viable and mature seeds to resume growth immediately after harvest under favourable for the germination of the concerned plant species is known as seed dormancy..

The period of dormancy varies from a few days to several years depending on the plant species.

Advantages:

1. Storage of seeds is prolonged, it is a survival mechanism
2. Seed can pass through adverse situation /conditions
3. Prevents the in-situe germination i.e., vivipary

Disadvantages:

1. No uniform germination
2. Difficult to maintain plant population
3. Interferes in seed testing procedure

Types of dormancy

Nikolaeva (1969 and 1977) classified dormancy into three broad class are as below;

- **Exogenous Dormancy**: Dormancy is due to some external features of the seed located outside the embryo-
    i. **Impermeability of seed coat to water**: due to seed coat structure, which is hard enough to restrict the entry of moisture into the seeds, thereby preventing seed germination. Ex: Malvaceae, Leguminoseae, Lilaceae
    ii. **Impermeability of seed coat to gases**; is related to the insufficient intake of oxygen by seeds due to impermeability of seed structure enclosing embryo. Ex: Graminaceae, fruit crops & forest trees

iii. **Mechanical resistances of seed coat**: growth of embryo is checked due to extremely hard seed/fruit structure such as seed coat, endosperm per carp etc., Ex: Acacia species.

iv. **Inhibitors present in seed coat/endosperm**: biochemical substances present in seed coat or endosperm block the germination of embryo. Ex: Iris app-inhibitors present in the endosperm,Barley- Aflotoxin, Squash-Dichlobenil Tomato-Feruline and Caffeie acid, All spp.- Coumarin

- **Endogenous dormancy**: This type of dormancy occurs due to internal factors that is present within the embryo
    i. **Incomplete embryo development**: due to an incomplete development of the embryo. In such cases, germination does not occur until the embryos develop to their normal size. Ex: Palmaceae, Amgnoliaceae
    ii. **Inhibitors present within the embryo**: Dormancy arises from metabolic blocks produced by biochemical substances called inhibitors present within the embryo. In such cases germination can commence only when these inhibitors are leached out of the embryo Ex: Xanthium, Fraximus

- **Combined Dormancy**: dormancy is produced by a combination of two or more factors which act in complementary fashion. Ex: Fraximus

Methods of breaking Dormancy

1. **Natural breaking of dormancy**: in nature dormancy terminates when embryo gets suitable environment such as adequate moisture, aeration and temperature. The impermeable seed coat present in many species became permeable due to the rupturing of softening action of natural agents like micro organism, high or low temperature, humidity fiber and abrasion due to wind or digestive tracts of birds and animals which feed on these seeds. Ex: Rhizoctonia damages seed coat
2. **Treatments to break Dormancy**: the various treatments for overcoming dormancy may be divided into the following three groups
    i. **Seed coat treatments**: These treatments aim at making hard seed coat permeable to water or gases either cracking or softening them. The process is

usually referred as scarification. These treatments are either physical or chemical in nature.

a. **Scarification:**

Acid scarification: treating seeds with concentrated acids like H_2SO_4 HCL etc.,

b. **Thermal scarification**: the seeds are treated with different temperatures and gases.

c. **Mechanical scarification**: The seed coat is damaged using mechanical means. Viz.,

*__Rubbing seeds__ on sand paper or by using mechanical scarifier.

*__Making small incision by piercing. Ex.__ Bitter gourd

*__Removing of entire seed coat__. Ex. rubber

ii. **Embryo treatments**:

a. **Stratification**: the incubation of seeds at a suitable low temperature (Usually 0-5 ^{0}C) over a moist substratum before transferring them to a temperature optimum for germination. Ex. Cherry (Prunus cerasus), Mustard and rape seeds

b. **High temperature treatment**: in some species, incubation at 40-50 ^{0}C for few hours to 1-5 days may be effective in overcoming dormancy. Ex. Rice seeds more than 15% seed moisture treated in hot water of 40 ^{0}C for 4-5 hours.

c. **Chemical treatments**: alternatively growth regulators or other chemicals may be applied to induced germination growth regulators commonly used GA3 (100ppm), kinetin (10-15ppm) and thio-urea (0.5-3%)

iii. **Miscellaneous approaches**:

a. Exposing seeds to light

b. Pressure treatment

c. Infra red radiation treatment

d. Magnetic treatment

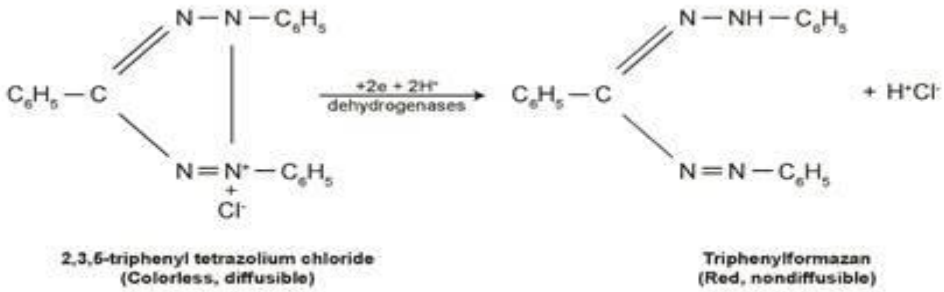
Topic:

SEED VIABILITY TEST: TZ TEST

TZ is a biochemical test and one of the quick methods to predict seed viability developed by Lakon (1942) in Germany.

Principle

In this biochemical test, living cells are made visible by reduction of an indicator dye. Indicator dye 2,3 5 Triphenyle tetrazolium chloride (soluble, colourless and diffusible) interferes with the reduction process of living cells and accepts hydrogenions from the hydrogenases enzyme. Red, stable and non-diffusible 2,3 5 Triphenyle Formazon (non-diffusible and red coloured) is produced in living cells. This makes it possible to distinguish the red coloured living part of a of seeds from the colour less dead ones.



Procedure

Viability test (TZ test): A test for viability that involves three steps:

1. Preconditioning (imbibitions)
2. Preparation and staining (sometimes cutting the seed and then soaking the seed in a 2,3,5 triphenyl tetrazolium chloride solution)

1. **Preparation**

a. Seeds are soaked in water overnight. They may be pre-moistened, in which case the seeds are allowed to imbibe water between a moistened germination paper blotter.

b. Seeds are then dissected, either longitudinally or transversely, with a scalpel so that the embryo is exposed to the tetrazolium chloride solution. One half of this seed is used for the test and the other half is discarded.

2. Staining

a. A solution of 2, 3, 5 triphyenyl tetrazolium chloride (A salt-soluble, colourless and diffusible) is added to water to form a colorless solution.

b. The seeds are placed in a 1% solution (for legumes and grasses that are not bisected), or a dilute 0.1% solution for bisected grasses and cereals.

c. Seed coats of legumes must usually be removed or peeled back before examination. Care must be taken to prevent breaking of radicles and other damage to the seeds.

Interpretation of TZ Test result:

Dehydrogenase enzymes present in living tissue reduce the tetrazolium chloride to formazan, a reddish, water-insoluble compound. This reaction occurs in or near living cells, which are releasing hydrogen in respiration processes. Depending on size, all seeds are examined under a microscope at 10-30 power. Larger seeds, such as peas, may be examined without a microscope. Analysts look for three things:

i. **Completely stained-** seeds treated as viable while partially stained/ unstained seed are evaluated as non-viable

ii. **While interpreting results** of TZ test, the areas of seeds which are vital importance in monocot & dicot are as follows-

Monocot:

a. The growing tip of embryonic axis- especially plumule

b. Point of attachment of embryo to the scutellum

c. Region of seminal root emergence (Radicle)

Dicot:

a. Radical & hypocotyle region

b. Cotyledons

c. The plumule region

Reporting of results

TZ test results are recorded as a percentage and are usually reported in the remarks section of the Certificate of Seed Analysis issued by the seed laboratory.

%age of Viability = No. Of stained seed/ total numbers of seed placed for TZ testing.

Accuracy

Properly conducted TZ and germination test results are generally in close agreement and within the range of normal sampling variation. Differences of 3% to 5% may be due to an unavoidable sampling variation error

Advantages

The major advantages of the tetrazolium test are:

a) Provides rapid evaluation: less than 20 h for most crops;
b) Allows identification of seed vigor level;
c) Diagnosis the cause(s) of seed deterioration;
d) Requires simple and inexpensive equipment;
g) Dormant seeds can also be evaluated.

Limitations

The major disadvantages of the tetrazolium test are:

a) Requires training and knowledge of seed structures and proper tetrazolium interpretation;
b) Is tedious due to the examination of individual seeds that requires patience and experience;
c) Shows neither the efficacy of chemical seed treatments nor their potential phyto-toxicity;

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