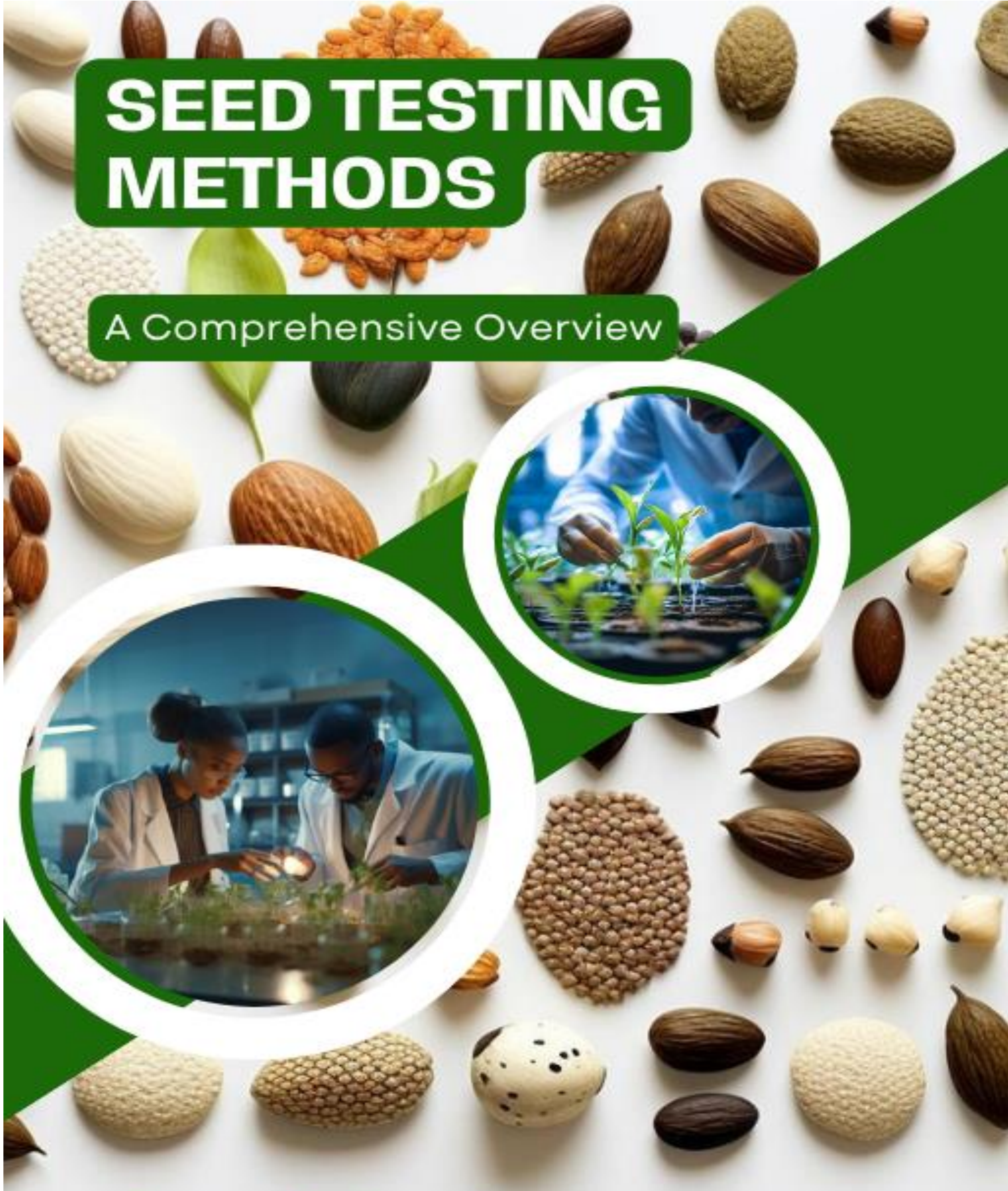
SEED TESTING METHODS

A Comprehensive Overview



Arnab Gupta

Seed Testing Methods:

A Comprehensive Overview

Arnab Gupta

Contents

Representative images generated through Midjourney.com

Introduction

Quality seed is the basic and most critical agricultural input determining plant stand establishment, growth, productivity and profitability. Seed testing enables objective assessment of seed quality parameters through systematic scientific examination of seed samples in a laboratory (AOSA, 2022). It serves as an integral tool for seed certification, regulatory control, trading, variety protection and performance prediction.

Reliable seed testing provides benefits throughout the seed value chain including:

1. Enabling seed producers make improvements based on quality feedback.
2. Guiding seed processing, conditioning, packaging and labelling.
3. Supporting seed distribution, marketing and price establishment.
4. Assuring quality to farmers and minimizing crop losses and risks.
5. Settling disputes regarding seed lot standards and values.
6. Tracking origin and checking claims of varietal identity and purity.

This manual provides comprehensive coverage of all key aspects of seed testing including:

- Role and organization of seed testing laboratories
- Seed sampling considerations and procedures
- Physical purity analysis methods and interpretation
- Germination testing protocols and evaluation
- Moisture testing principles and techniques

- Other viability, vigour and health testing assays
- Quality control and precision measures
- Automation and technological advancements
- Scope for improvements in seed testing systems

Understanding seed testing concepts and procedures enables stakeholders to derive full benefits from this quality control tool that forms the gateway to high productivity through quality seed.

Organization of Seed Testing Laboratories

Seed testing involves specialized techniques and requires expert analysts trained to follow standardized international procedures prescribed by bodies like the International Seed Testing Association (ISTA, 2022) and Association of Official Seed Analysts (AOSA, 2022). Testing is performed in accredited laboratories with dedicated sections for:

1. **Physical Purity Analysis** Physical Purity Analysis is a critical process in seed technology. It involves the examination of a representative seed sample to ascertain the varietal identity by physical characteristics. This assessment also checks for the presence of inert matter, such as stones, soil, broken seeds, and other crop or weed seeds, which should not exceed the maximum allowable limits. The process is vital for ensuring the seed lot meets the specified standards for purity, which is an indication of the quality of the seed cleaning operation. This analysis helps in maintaining seed quality by preventing the spread of undesired plant material and ensuring the accurate labeling of seed packages (ISTA, 2021).
2. **Moisture Testing** Moisture content is a significant factor affecting seed storability, germination, and vigour. Moisture Testing is conducted to determine the water content in a seed sample. High moisture levels can lead to increased microbial activity, potentially causing seed deterioration and reducing shelf life. Conversely, very low moisture levels can damage seed viability. Moisture testing is typically performed using methods such as oven drying, which provides a basis for adjusting storage conditions to maintain seed quality over time (AOSA, 2020).
3. **Germination Testing** Germination Testing is a standard procedure to predict the potential for field emergence of seeds under optimal conditions. This test measures the percentage of seeds that can germinate and produce normal seedlings, which is crucial for determining sowing rates and expected crop yields. Germination tests are conducted under controlled environmental

conditions that provide the best possible scenario for seedling development, hence serving as a benchmark for seed viability (ISTA, 2021).

4. **Vigour Testing** Vigour Testing goes beyond germination tests by evaluating the ability of the seed to perform in suboptimal conditions. It assesses the seedling growth characteristics and stress tolerance, providing insights into the potential field performance of a seed lot under a range of environmental stresses. Vigour tests include accelerated aging, cold tests, and electrical conductivity tests, each examining different aspects of seed performance and predicting the seed's capacity to establish a healthy crop under various field conditions (AOSA, 2020).
5. **Seed Health Testing** Seed Health Testing is designed to detect the presence of seed-borne pathogens, such as bacteria, fungi, viruses, and nematodes, that can transmit infections to the next plant generation. This testing ensures that seeds are free from diseases that could otherwise spread across large agricultural areas, causing significant economic losses. Methods used in seed health testing include blotter tests, agar plate tests, and PCR-based assays, which help in the identification and quantification of pathogens within seed lots (ISTA, 2021).
6. **Biochemical Testing** Biochemical Testing in seeds measures the viability parameters that are indicative of the metabolic state of a seed and its ability to germinate and develop into a healthy plant. Such parameters include enzyme activity, which is crucial for the germination process, electrical conductivity, which indicates membrane integrity, and respiration rate, which reflects metabolic activity within the seed. These tests can provide a more detailed understanding of seed viability and vigour, supplementing the information obtained from physical, germination, and vigour testing (ISTA, 2021).

References:

- International Seed Testing Association (ISTA). (2021). International Rules for Seed Testing.
- Association of Official Seed Analysts (AOSA). (2020). Seed Vigour Testing Handbook.

The laboratories maintain optimal infrastructure, equipment calibration and trained personnel adhering to precise testing protocols and quality standards. This ensures accurate, reproducible test results and data integrity. External check samples and audits validate reliability.

As an example, in India, the Central and State Seed Testing Laboratories form the national seed testing system responsible for quality assurance under the regulatory framework of the Seeds Act, 1966 and Seed Rules, 1968. The State Seed Testing Laboratories (SSTLs) are ISO 9001 certified and accredited by the National Accreditation Board for Testing Calibration Laboratories (NABL) which accredits labs under the ISO IEC 17025:2005 EC, for reliability. **This basic structure is applicable to any seed testing laboratory and ISO 17025 is the primary guideline for accreditation for STLs.**

Objectives and Importance of Seed Testing

The fundamental goals served by seed testing include:

1. Determining if seed samples meet prescribed quality standards for certification and truth-in-labelling (Elias and Copeland, 1997).
2. Providing information on seed viability, vigour, storage needs, dormancy, planting value and field performance expectations.
3. Detecting quality defects and probable causes to guide seed conditioning procedures.
4. Supporting quality regulation and commerce through objective data for decision making.
5. Settling disputes regarding seed lot standards and value.
6. Tracking origin and verifying varietal identity and purity claims.
7. Monitoring quality deterioration for regeneration and disposal decisions.

Reliable seed testing safeguards farmers against poor quality planting material susceptible to poor germination, delayed emergence, inadequate stands and low productivity. It prevents introduction and spread of low quality seeds and diseases to new areas. Test data supports seed distribution, pricing and trade based on transparent quality benchmarks. Seed testing is thus pivotal for seed quality assurance.

Seed Sampling and Submitting Protocols

Seed testing accuracy fundamentally relies on representative sampling. Small submitted samples are tested to predict the field performance of large seed lots. Meticulous sampling is vital as quality is seldom uniform within a seed lot and tests are sensitive to sampling errors. Key steps in seed sampling include:

1. Defining the Seed Lot

The seed lot must be physically identifiable and distinct based on uniform origin, variety, markings, container etc. Seed lot size and heterogeneity determine subsequent sampling protocols.

In the context of seed certification and testing, both the International Seed Testing Association (ISTA) and the Association of Official Seed Analysts (AOSA) provide definitions and standards for a "seed lot." A seed lot is generally defined as a quantity of seed that is uniform in its characteristics, meaning it is identified by a unique set of descriptors, such as:

- Origin (where the seed was grown)
- Variety (the specific type of plant)
- Year of production
- Specific field or production area designation
- Processing history
- Any treatments applied

According to ISTA, a seed lot should be "uniform in composition and so far as can be judged by the information available, free from any pests, diseases or other organisms which may affect its sowing quality" (ISTA, 2021).

However, A seed lot would represent any quantity of agricultural seeds upto a maximum of 20,000 kilogrammes for seeds of the size of rice or larger (except maize seed, seed potato, sweet potato, yams, taro and chow-chow for which the maximum size of the lot may be 40,000 kilogrammes) and 10,000 kilogrammes for seeds smaller than rice subject to a tolerance limit of 5.0%. The quantities in excess of the above maximum limits shall be sub-divided and separate lot identification shall be given. See the table in Annex 2 for the maximum seed lot sizes

2. Collecting Primary Samples

Multiple primary samples are drawn evenly across the entire seed lot using triers or manually. Sample number, size and distribution depends on lot volume and heterogeneity.

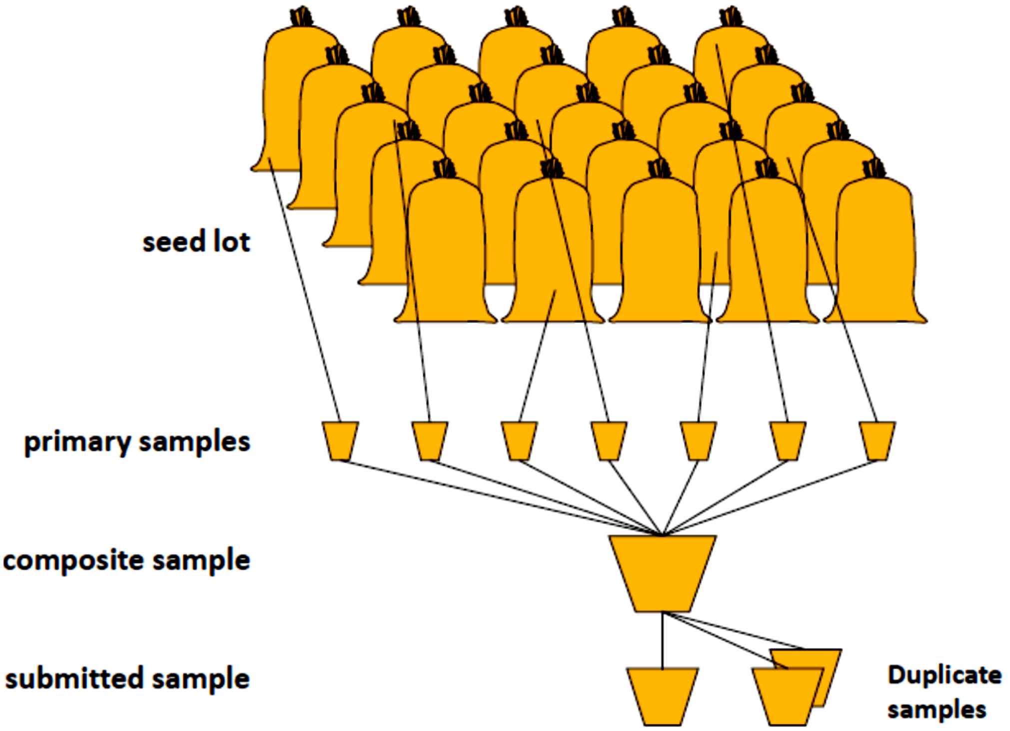
Sampling intensity: In accordance with ISTA standards, the sampling intensity—meaning the number of samples collected from a seed lot for testing—depends on the size of the seed lot and the type of seed. The ISTA Rules specify maximum lot sizes for each species. For most agricultural and vegetable seeds that are smaller than wheat, including many grass species, the maximum lot size is typically 10,000 kilograms. For larger seeds, the maximum lot sizes can be larger, ranging from 20,000 to 30,000 kilograms. However, the lot sizes for different types of crops, like trees and flowers, can vary significantly (see Annex 2) .

The sampling must represent the entire lot, and the process should be carried out only when the seed is ready to be shipped, after any treatments or rebagging. This ensures that the sample reflects the seed lot as it will be received by the importer. It's important to note that any treatment of the seeds after testing will invalidate the certificate since the seeds no longer represent the tested sample.

For specific sampling procedures, including the minimum number of primary samples required for heterogeneity testing, ISTA offers tools such as the ISTA Sampling Calculator. This tool helps in calculating the number of samples based on various variables such as seed type, container size, and packaging.

3. Preparing Composite Sample

All primary samples are combined and thoroughly mixed to obtain a homogeneous composite sample representing the seed lot.



4. Obtaining Submitted Sample

The submitted sample of specified size for different tests is derived from the composite sample through methods like repeatedly halving/quartering or withdrawing random portions.

Every crop, based on the seed size, has a standard submitted sample size.

- **1000 grams** for maize, cotton, groundnut, soybean, and species of other genera with seeds of a similar size.
- **500 grams** for sorghum, wheat, paddy, and species of other genera with seeds of similar size.
- **250 grams** for Beta and species of other genera with seeds of similar size.
- **100 grams** for bajra, jute, and species of all other genera.
- **250 tubers/planting stakes/roots/corns** for seed potato, sweet potato, and other vegetatively propagating crops

5. Packaging, Labelling and Submission

Submitted samples are securely packed to avoid damage or contamination in transit and accurately labeled with details like variety, lot number, test requirements, sender etc. before prompt submission to the testing laboratory. When seeds are to be sent to the lab for seed moisture content test also, they are packed in metal tins, aluminum foil pouches or any other moisture vapor proof packets.

6. Working Sample Preparation

The laboratory prepares uniform working samples from the submitted samples for conducting the various tests.

Following standardized sampling and sample reduction protocols minimizes errors and enhances test result reliability. The sample size requirements prescribed by ISTA for various crops and tests serve as international benchmarks.

Food and Agriculture Organization
of the United Nations

Manual methods

2

Spoon method – **comprising a tray, a flat-edged spatula and a flat spoon with vertical sides. Appropriate for species smaller than wheat.**

Modified halving – **comprising a tray fitted with a grid of cubical cells that are alternately open at both ends or closed at the bottom. Allows halving of the sample, but no other division.**

Hand halving method – **comprising a flat edged spatula and a straight-edged instrument (e.g. knife or ruler). Appropriate for chaffy seed species, as listed in the ISTA Rules.**

- ✓ *mechanical reduction methods are often recommended*
- ✓ *Hand reduction methods are more suitable. in specific situations (very chaffy seed, unprocessed seed, seed health testing),*

FAO/AfricaSeeds Validation Workshop
Seeds Toolkit

Abidjan, Côte d'Ivoire
14-18 November, 2016

Preparing a working sample in a seed testing lab involves three key steps:

1. Receiving and recording:

- The submitted seed sample arrives at the lab and is documented with details like species, weight, and test requirements.

2. Mixing and dividing:

- The entire sample is thoroughly mixed to ensure uniformity.
- Mechanical dividers (like Boerner dividers) or manual methods (like spreading and splitting) are used to reduce the sample size to the required amount for specific tests. This process might be repeated to achieve the desired working sample size.

3. Sub-sampling (optional):

- For some tests, specific sub-samples may be needed (e.g., counting for germination tests). These are carefully drawn from the working sample following specific protocols to maintain representativeness.

Key features:

- Maintaining representativeness: Each step aims to ensure the working sample accurately reflects the entire seed lot.
- Standardized procedures: Labs follow specific ISTA or national/regional standards for mixing, dividing, and sub-sampling to obtain reliable results.
- Equipment use: Mechanical dividers like soil type/ Riffle dividers or Boerner types are common for efficient and unbiased sample reduction. Alternatively, hand-halving can also be used.(see fig)

Physical Purity Analysis

Physical purity refers to the percentage by weight of pure seeds of the specified kind in a seed lot along with data on contaminants like inert matter, weed seeds, other crop seeds etc. **Determining sample purity is the first step in seed testing with the following workflow**:

1. Drawing Working Sample

The prescribed working sample size is drawn from the submitted sample based on crop type. For large seeds like cereals and legumes, the working sample size is around 10-100 grams. For small seeds like vegetables it may be only 1-2 grams. **But the whole working sample of the prescribed size must be used for physical purity assessment**. See Anexe 3 for working sample size for physical purity testing.

2. Fractionating Sample Components

The working sample is fractionated by physical separation into pure seeds, inert matter, other crop seeds, weed seeds and damaged/deteriorated seeds.

3. Weighing the Fractions

Each fractionated component is carefully weighed to determine the percentage by weight it constitutes in the working sample. Weighing is performed to the prescribed number of decimal places based on sample size for accuracy.

4. Identification of Contaminants

Seed contaminants like individual weed species and other crop seeds are identified and enumerated as their presence above threshold levels may affect field performance.

5. Calculating Percentages

Using the weights of each fraction and total sample, percentages of pure seeds and contaminants are calculated using the formula:

Percentage of component = Weight of component x 100 / Total weight of all components

6. Duplicate Tests and Tolerances

Tolerances are statistical methods used in seed testing to assess the acceptability of results based on expected variations. These variations can occur within replicates of the same test, between different tests of the same seed lot, and even between different testing laboratories. In seed testing, tolerances are particularly important for verifying the accuracy and reproducibility of results.

By comparing the difference between two results and comparing this difference to statistically predetermined values, we can determine if the results fall within acceptable limits. Standardized tolerance tables exist for specific tests, such as physical purity and germination, allowing us to evaluate both the reproducibility and accuracy of our seed testing procedures within a laboratory. Refer to Annex 4 for these specific tolerance tables.

Note:

Purity analysis results determine the need for seed conditioning besides verifying if statutory quality standards are met. Seed certifying agencies utilize purity data to decide on seed lot approval and labeling.

Germination Testing

1. Definition

According to botanists, germination is the sprouting and emergence of the radical (root). According to seed technologists, germination is the emergence and development of the seedling with all essential structures which indicate the ability to develop into a normal plant under favorable conditions.

2. Objective

Germination testing indicates the ability of seeds to emerge and develop into normal plants, reflecting their viability and planting value. The results provide information on whether seeds are suitable for sowing.

3. Importance of Germination Testing

- Provides information on viability and planting value of seed lot prior to sowing season.
- Results should be reproducible and acceptable to end users for decision-making on sowing.
- Serves as an index of capacity to produce normal, healthy seedlings that can survive and grow to maturity under optimal conditions.
- Allows estimation of potential field emergence and crop stand.

4. Equipment and Materials Needed

- Seed germinator: An enclosure with controls for maintaining consistent temperature, humidity and lighting conditions.
- Thermometer: For monitoring temperatures inside germinator. Use liquid-in-glass or digital thermometers accurate to 0.5°C.
- Vacuum counter: For quickly and accurately counting seeds.
- Seed scarifiers: For breaking hard seed coats prior to testing. Can use sandpaper, knives, clippers, etc.
- Soil and sand boxes: For holding substrate during testing. Use inert plastic or glass boxes.

- Sterilizers: For sterilizing equipment and materials. Can use hot water, detergent, autoclave, UV light, etc.
- Sprinkler: For moistening substrate during testing. Use fine mist nozzle to avoid seed displacement.
- Magnifier: For inspecting small seeds. Use 5X to 10X magnification.
- Blotting paper: Absorbent paper providing substrate and moisture. Use high wet strength paper.
- Petri dishes: For holding seeds during testing. Use 90 to 150 mm dishes.
- Glass plates: Also used to hold seeds during testing. Use 10-20 cm square plates.
- Polyethylene packets: To maintain humidity during testing. Seal plates/dishes in packets.
- Tray racks/holders: To arrange test units in germinator. Use plastic or stainless steel.
- BOD incubator: For maintaining humidity and darkness.
- Sample trays: To hold packets, plates, or dishes during testing.
- Hygrometer: To monitor humidity levels inside germinator. Digital or analog ≤ 2% accuracy.
- Test tubes: For soaking paper or pre-chilling/heating seeds.
- Measuring cylinders: For preparing solutions and measuring reagents.
- Conical flasks: For soaking and rinsing paper.

5. Substrates Used

The medium on which seeds are grown during testing varies by crop. Common substrates are:

- **Blotting Paper**: Should be free of toxic substances. The paper is cut to size for plates or dishes and soaked in water for 2-4 hours before use to moisten evenly and remove any water-soluble toxins. Quality standards:
    1. No microbial infection
    2. Good water holding capacity
    3. Good wet mechanical strength
    4. Neutral pH
- **Sand**: Sand particles provide structure and moisture retention. Wash thoroughly before use to eliminate any phytotoxic compounds. The amount of water added will depend on the water holding capacity of the sand, usually around 50-60%.

- **Soil**: Used in cases where phytotoxicity symptoms are observed with sand. Provides similar structure and moisture, reduced risk of phytotoxins.

Procedure for germination testing

Drawing Working Sample: Appropriate working sample size in terms of number of seeds tested depends on seed size and shape. For large seeds like cereals and legumes, 4 replicates of 100 seeds are tested. Small seeds may require 8-16 replicates of 25-50 seeds for accuracy. **The pure seed fraction separated after a Physical Purity test is the working sample for a germination test.**

Requisite number of seeds are used for the germination test from that fraction.

400 seeds is the minimum germination test sample size and this can be distributed into required number of replications based on seed size and test method.

Eg 4x100; 8x50

1. **On Top of Paper**
    - Select absorbent blotting paper and cut sheets to fit the size of the glass plates.
    - Soak the paper in water for 2-4 hours before testing. This helps evenly moisten the paper and leach out any water-soluble toxic substances that may be present.
    - Thoroughly wash the glass plates that will hold the seeds and paper. Use soap and tap water to remove any residue, oils, or debris on the plates.
    - Rinse the plates well with distilled water to remove any soap residue. Dry completely.
    - Place the pre-soaked blotting paper sheets on top of the cleaned glass plates.
    - Use sterilized forceps to carefully transfer seeds onto the top of the paper on each plate. Space the seeds evenly, at least 1-5 times the seed width apart. Typical number per plate is 15-25 seeds.
    - Cut small strips of the blotting paper and moisten them. Place these strips over the seeds to cover them.
    - Anchor the paper strips in place by clipping the ends with binder clips. Secure the clips on each side with rubber bands.
    - Put the glass plates with seeds into polyethylene packets to retain humidity. Place packets upright in trays.
    - Add a small amount of water to each packet to keep the paper moist. Take care not to oversaturate.
    - Place the trays into the germination chamber set at the specified temperature for the crop.
    - On day 5 of testing (first count day), remove trays and count the number of normal and abnormal seedlings on each plate.
    - Return trays to germinator and continue daily counts until final day.
    - Record counts each day and calculate percent germination.
2. **Between Paper Layers**

- Follow the same procedure as above, but place seeds between two layers of the pre-soaked blotting paper in step 6 instead of on top.

3. **Pleated Paper**

- Follow the same procedure as above, but in step 6 fold the pre-soaked paper into 10-15 pleats and place seeds along the folds instead of on top.

4. **On Top of Sand**
    - Wash sand thoroughly to remove any phytotoxic compounds prior to testing.
    - Adjust moisture content of sand to 50-60% of its water holding capacity, depending on seed size.
    - Distribute seeds evenly on the top of the moistened sand in trays.
    - Follow the same procedure as in steps 7-14 above.
5. **Between Sand Layers**
    - Fill trays partway with pre-moistened sand.
    - Distribute seeds evenly in a layer on top of the sand.
    - Cover seeds with 1-3 additional layers of moistened sand.
    - Follow the same procedure as in steps 7-14 above.

6. **In Soil**

- Plant seeds on top of or between layers of soil prepared at suitable moisture content. Follow the same procedure as in steps 7-14 above. Soil is used when phytotoxic effects are observed with sand substrate. **However, since soil cannot be standardized, it is not recommended by ISTA as a standard germination substrate**

Temperature and incubation

Proper temperature is critical for normal germination and seedling development during testing. Optimal temperatures vary by crop and should be determined from protocols. The general range suitable for many seeds is 15°C to 30°C, with 20-25°C appropriate for a variety of crops. Temperatures must remain consistent throughout testing, as fluctuations above 1-2°C can significantly impact results. Use of calibrated germinators or incubators capable of precisely maintaining set temperatures is recommended.

Some species require alternating low-high temperatures during testing. A 16 hour lower temp period followed by an 8 hour higher temp period should be maintained.

Proper incubation involves maintaining ideal conditions including stable temperatures, high 95-100% humidity to prevent drying, and appropriate light/darkness. Most seeds germinate best in darkness. Minimize light exposure using dark incubators and tray lids. Provide low intensity illumination ≤1250 lux during an 8 hour photoperiod for light-requiring seeds.

During incubation, samples should be arranged evenly spaced apart on trays to minimize temperature variations. Contamination can be avoided by proper sterilization and aseptic technique.

7. Seedling Evaluation Criteria

Carefully evaluate each seedling on every count day and classify as follows:

A. Normal Seedling

- Capacity for continued growth and development
- Well developed essential structures:
  - ✓ Root system
  - ✓ Hypocotyl and epicotyl
  - ✓ Coleoptile with enclosed leaf (monocots)
  - ✓ Cotyledons fully emerged (dicots)
- Well developed plumule and roots

B. Abnormal Seedling

- ✓ Weak, stunted, or deformed plumule
- ✓ Weak, stunted, missing, or decayed roots
- ✓ Damage or decay in essential structures
- ✓ Limited potential for continued growth

C. Other Abnormalities

- ✓ Damaged: Missing cotyledons, cracks, lesions
- ✓ Deformed: Twisted or swollen structures
- ✓ Decayed: Diseased, rotten tissue
- ✓ Inhibited: Lack of root/shoot development

D. Hard, Fresh and Dead Seeds

- Hard: Impermeable seed coats, remain hard at end of test
- Fresh: Intact, firm, viable, but failed to germinate
- Dead: Non-viable, neither firm nor viable

8. Interpreting Results

- Count only normal seedlings each day
- Calculate percent germination of each replicate
- Determine average percent germination across replicates
- Compare to certification standards for the crop
- Results on day 5 or first count indicate seedling vigor
- Testing can be truncated once certification standard is reached

9. Necessity of Re-Testing

Germination testing should be repeated when:

- Dormancy, phytotoxicity, or infections are present
- Many fresh ungerminated seeds remain
- Test conditions are incorrect or seedling evaluation is inconsistent
- Variation among replicates exceeds tolerance
- Errors occur in recording or calculations
- Results between first and second test do not match statistically

10. Key Factors for Reliable Results

To achieve consistent, reliable results, four key factors must be controlled:

1. **Seed Spacing**
    - ✓ Space seeds at 1-5x their width to prevent crowding and interaction between seedlings.
    - ✓ Overcrowding can impact germination rates and seedling development.
2. **Moisture Levels**
    - ✓ Add sufficient water to meet crop requirements without saturating substrate.
    - ✓ Both low and excess moisture can reduce germination.
    - ✓ For paper, humidity should be near saturation to prevent drying.
    - ✓ Monitor moisture

Calculation and Expression

The number of normal seedlings in each replicate is recorded. The average of replicates gives the percentage germination which is **rounded off** and reported. So a final result of 4 replicates showing 87, 89, 82, 85 will have an average of 85.75 so the final report will be 86%. Same will be the reporting of the abnormal, dead, hard and fresh seeds

The replicates must be tested against the Tolerance tables to ascertain the result accuracy

Look into Annex 5 for the standards of temperature, pretreatments, count duration etc.

Other Viability Testing

Besides standard germination, seed viability and planting potential is assessed through tests like:

entioned:

1. **Tetrazolium Test**:
    - **Description**: The tetrazolium test is a biochemical assay based on the activity of dehydrogenase enzymes present in living tissues. These enzymes reduce colorless tetrazolium salts to formazan, a red-stained compound, indicating viable tissues.
    - **Purpose**: It is commonly used for rapid viability estimation in seeds and for analyzing seed damage. By staining viable tissues, it helps distinguish between viable and non-viable seeds.
    - **Application**: Seeds are soaked in a tetrazolium salt solution and incubated under controlled conditions. Viable tissues absorb the solution and produce a red stain, while non-viable tissues remain unstained.
    - **Reference**: Lakon (1949) is cited as a foundational work in this field.
2. **Excised Embryo Test**:
    - **Description**: In this test, embryos are surgically removed from seeds and germinated separately from the seed coat. This method allows for the evaluation of embryo germination potential independently of any seed coat-related dormancy.
    - **Purpose**: It helps distinguish between seed dormancy (caused by factors in the seed coat) and embryo dormancy (inherent to the embryo itself).
    - **Application**: The embryos are placed on a germination medium and monitored for germination. Any delay or difference in germination compared to intact seeds indicates the presence of seed coat-imposed dormancy.
3. **Topographical Tetrazolium Test**:

  - **Description**: This test is a variation of the tetrazolium test where the staining pattern is used to identify specific areas of damage or weakness within the seed.
  - **Purpose**: It enables the localization of weak, damaged, or dead tissues within seeds, providing insights into the extent and nature of seed damage.
  - **Application**: Seeds are soaked in a tetrazolium solution, and the staining pattern is examined under a microscope. Variations in staining intensity or patterns can indicate areas of concern, such as mechanical damage or disease.

4. **Radiographic Test**:
   - **Description**: This test involves using X-ray imaging to visualize the internal structure of seeds. It is particularly useful for detecting mechanical damage, insect damage, or developmental defects that may affect seed viability.
   - **Purpose**: It provides a non-destructive method for assessing internal seed quality, which may not be apparent from external inspection alone.
   - **Application**: Seeds are exposed to X-rays, and the resulting images are analyzed for abnormalities or irregularities in seed structure.
5. **Indirect Tests**:
   - **Description**: Indirect viability tests involve measuring various biochemical markers or physiological processes associated with seed viability.
   - **Purpose**: They provide insights into seed metabolic activity, which can be indicative of viability. Common indirect tests include measuring electrical conductivity, respiration rate, ATP content, amylase activity, and DNA/RNA quantitation.
   - **Application**: These tests are used to predict seed viability and assess seed physiological status. They are particularly valuable for research purposes and in cases where direct viability tests may not be feasible or practical.

These tests, although not widely used in routine seed testing, offer valuable tools for assessing seed viability and understanding seed physiology in more detail. They can complement traditional viability assays and provide additional insights into seed quality and performance.

Moisture Testing

Introduction

Every seed contains a certain amount of moisture after harvesting. Determining the moisture content of seeds is important for several reasons:

- To track moisture levels at different crop growth stages
- To identify ideal seed samples by comparing to standard moisture levels
- Moisture content strongly influences seed viability and shelf life

This chapter will cover the key aspects of seed moisture testing, including:

- Equipment needed
- Test procedures
- Calculation of moisture percentage
- Maximum allowable moisture for certification
- Importance of proper moisture levels

Equipment Needed

The main equipment used in moisture analysis of seeds includes:

- **Oven** - Stainless steel cabinet with hot air circulation up to 200°C. Used for drying seeds at desired temperatures.
- **Desiccator** - Thick glass container with air-tight lid. Contains drying agents like silica gel. Used for moisture-free cooling after oven drying.
- **Seed grinder** - Grinds seeds evenly without overheating. Allows adjustment of fineness.
- **Containers** - Made of glass or stainless steel to hold seeds for drying.
- **Sieves** - Wire mesh sieves of 0.5mm, 1mm and 4mm sizes to prepare samples.
- **Analytical balance** - Sensitive scale for weighing samples and containers.

Test Procedures

There are two main methods for determining seed moisture percentage:

1. Air Oven Method

This involves drying seed samples at either 103°C (low temp) or 130°C (high temp) for specified durations based on the crop type. The loss in weight after drying is used to calculate moisture percentage.

2. Electric Moisture Meter Method

Rapid electronic meters work by measuring conductivity or dielectric properties of the seeds. Results can be obtained within minutes.

For large-seeded crops, samples need to be ground into fine powder before testing to get accurate readings. If moisture content is very high, a pre-drying step at lower temperature may also be required.

Calculation of Moisture Percentage

The moisture content (M) is calculated using the formula:

M = [(Initial weight - Oven dry weight) / Initial weight] x 100

For pre-dried samples, the moisture percentages from both stages are used to determine the original moisture content.

Samples are tested in duplicates or triplicates and results averaged. Tolerances of 0.2-0.5% are prescribed between duplicates. Moisture data is vital for storage, packing and labeling decisions.

Maximum Seed Moisture for Certification

The seed certification agency specifies the maximum moisture percentage allowed for label categories like foundation and certified seeds of different crops.

For example, the limit is 13% for paddy, 12% for wheat and 10% for soybean in open containers. Lower limits apply when using moisture-proof packaging.

Importance of Seed Moisture

- Proper moisture content is critical for maintaining seed viability in storage. High moisture leads to accelerated deterioration while too little moisture causes dryness damage.
- Monitoring seed moisture and drying when required ensures high quality planting material with optimal shelf life and germination capacity.
- Determining and controlling moisture content is an essential step in seed processing and storage. This chapter covered the standard methods, calculations and equipment needed for accurate moisture testing along with permissible limits and significance of seed moisture. Proper moisture management improves seed lot quality, storability and performance in the field.

Vigor Testing

Seed vigour evaluation is fundamental in determining seed quality and potential field performance. This chapter aims to provide a detailed exploration of five key vigour testing methods, elucidating their objectives, methodologies, and significance in seed quality assessment and crop management.

1. Seedling Growth Tests:

Objectives: The primary objective of seedling growth tests is to assess the vigor of seeds by evaluating their ability to germinate and develop into healthy seedlings under controlled conditions. Specific objectives include determining germination rate, root and shoot length, and seedling biomass, which collectively indicate early seedling vigor and growth potential.

Methodology:

- Select a representative sample of seeds.
- Germinate seeds in a controlled environment with optimal temperature, humidity, and light conditions.
- Monitor seedling emergence and growth parameters such as root and shoot length, biomass accumulation, and germination rate over a predetermined period.
- Calculate indices such as vigor index or germination index to quantify seedling vigor and growth performance.

Significance: Seedling growth tests provide valuable insights into seed vigor and early seedling performance, enabling seed producers and researchers to identify high-vigour seed lots for optimal crop establishment and productivity.

2. Tetrazolium Vigour Test:

Objectives: The tetrazolium vigour test aims to assess seed viability and vigor by evaluating tissue viability and membrane integrity. It provides insights into the physiological status of seeds and helps identify viable and vigorous seeds for planting.

Methodology:

- Treat seeds with tetrazolium solution, which is metabolized by living tissues to form a red-stained product. Living tissues have the enzyme dehydrogenase, which is detected by this salt by staining living tissues red

- Incubate treated seeds under suitable conditions (Dark, 30^0C) to allow tetrazolium staining to occur.
- Examine stained seeds under a microscope to assess staining patterns, indicating viable and non-viable tissues.
- Calculate viability indices based on staining intensity and distribution to quantify seed vigor.

Significance: The tetrazolium vigour test is a rapid and reliable method for assessing seed viability and vigor, enabling seed producers to select high-quality seeds with optimal germination potential.

3. Conductivity Test:

Objectives: The conductivity test evaluates seed membrane integrity by measuring electrolyte leakage, which indicates membrane deterioration and seed deterioration. It helps identify seeds prone to deterioration and storage problems. So, more is the electrical conductivity measured of the seed leachate, more deteriorated is the seed lot

Methodology:

- Soak seeds in distilled water to extract electrolytes from damaged seeds.
- Measure electrical conductivity of the soaking solution using a conductivity meter.
- Calculate electrolyte leakage as an indicator of seed membrane integrity and deterioration.

Significance: The conductivity test serves as a sensitive indicator of seed membrane integrity and deterioration, allowing for early detection of seed quality issues and storage problems.

4. Stress Tolerance Tests:

Objectives: Stress tolerance tests subject seeds to adverse environmental conditions such as cold, heat, or moisture stress to assess their ability to withstand and recover from stress. These tests help identify seeds with superior stress tolerance and resilience.

Methodology:

- Expose seeds to controlled stress conditions such as temperature extremes, water deficit, or salt stress.
- Monitor seed germination and seedling growth under stress conditions.
- Assess seedling recovery and vigor following stress exposure.

Significance: Stress tolerance tests provide valuable insights into seed resilience and adaptability to adverse environmental conditions, enabling seed producers to select stress-tolerant varieties for improved crop performance and resilience.

5. Accelerated Aging Test:

Objectives: The accelerated aging test simulates aging conditions by subjecting seeds to high temperature and humidity, accelerating deterioration reactions and assessing seed longevity and vigor. It helps predict seed storage life and identify seeds prone to premature aging.

Methodology:

- Place seeds in a humid chamber at elevated temperature for a specified duration.
- Monitor seed germination and vigor following accelerated aging treatment.
- Assess seed viability and vigor based on germination percentage and vigor indices.

Significance: The accelerated aging test is a valuable tool for predicting seed storage life and identifying seeds with optimal vigor and longevity, aiding in seed quality assessment and storage management.

Each vigor testing method offers unique insights into seed quality and performance, facilitating informed decision-making in seed production, storage, and crop management. By understanding the objectives, methodologies, and significance of these tests, seed producers and researchers can effectively evaluate seed vigor and enhance crop productivity and resilience.

Seed Health Testing

Introduction:

Seed health testing is a cornerstone of international seed trade, ensuring the safe exchange of seeds across borders while safeguarding global agriculture from the spread of devastating diseases. As global trade in agricultural commodities continues to expand, the importance of robust seed health testing methods in meeting **international phytosanitary standards** and quarantine regulations cannot be overstated. This chapter explores the pivotal role of seed health tests in facilitating seamless seed trade and compliance with stringent quarantine requirements, highlighting their significance in preserving crop productivity, biodiversity, and food security on a global scale.

1. Blotter Method:

Principles: The blotter method involves incubating seeds on moist blotting paper to promote fungal growth. Fungal hyphae emerging from the seeds are observed microscopically to detect surface and internally seed-borne fungi.

Procedure:

- Place seeds on moist blotting paper in petri dishes.
- Incubate under controlled conditions to promote fungal growth.
- Monitor seeds regularly for the emergence of fungal hyphae.
- Identify and characterize fungal colonies using microscopy or molecular techniques.

Applications: The blotter method is widely used for routine screening of seed lots for fungal contamination, providing valuable insights into seed health and potential disease risks.

2. Agar Plate Method:

Principles: In the agar plate method, seeds are plated on specific fungal culture media to encourage the growth of pathogenic fungi. Colonies that develop on the media indicate the presence of seed-borne pathogens.

Procedure:

- Plate seeds on agar plates containing selective fungal culture media.
- Incubate plates under optimal temperature and humidity conditions.
- Examine plates regularly for the presence of fungal colonies.
- Identify and characterize fungal isolates using morphological or molecular techniques.

Applications: The agar plate method offers high specificity and sensitivity for detecting seed-borne pathogens, making it suitable for accurate disease diagnosis and pathogen identification.

3. Seed Wash Test:

Principles: The seed wash test involves washing seeds in water or buffer solutions to isolate microbial inoculum for subsequent analysis. This method allows for the extraction and concentration of pathogens present on the seed surface.

Procedure:

- Wash seeds in sterile water or buffer solution to dislodge microbial contaminants.
- Collect the wash water containing microbial inoculum.
- Perform microbial assays or molecular analyses to detect and identify pathogens present in the wash water.

Applications: Seed wash tests are valuable for assessing the microbial load on seed surfaces and detecting pathogens that may not be detected using traditional methods. This method is particularly useful for screening large seed lots for microbial contamination.

4. Grow-Out Test:

Principles: The grow-out test involves germinating seeds and growing seedlings under controlled conditions to evaluate disease symptoms visually or through immunoassays. This method allows for the detection of latent infections and systemic diseases.

Procedure:

- Germinate seeds in sterile growth media or soil under controlled environmental conditions.
- Monitor seedlings for the development of disease symptoms, such as wilting, necrosis, or stunting.
- Perform immunoassays or molecular tests on symptomatic seedlings to confirm the presence of specific pathogens.

Applications: The grow-out test provides a comprehensive assessment of seed health by evaluating seedling vigor and disease susceptibility. This method is useful for detecting latent infections and assessing the effectiveness of seed treatment strategies.

5. Molecular Methods:

Principles: Molecular methods such as PCR (Polymerase Chain Reaction) and LAMP (Loop-Mediated Isothermal Amplification) allow for rapid and sensitive detection of seed-borne pathogens by amplifying target DNA or RNA sequences.

Procedure:

- Extract nucleic acids from seeds or seed wash samples.
- Perform PCR or LAMP assays using specific primers and probes targeting pathogen DNA or RNA.
- Analyze amplification products using gel electrophoresis or real-time PCR instruments.
- Interpret results based on the presence or absence of pathogen-specific amplicons.

Applications: Molecular methods offer high specificity and sensitivity for detecting seed-borne pathogens, allowing for rapid and accurate disease diagnosis. These techniques are particularly useful for detecting low levels of contamination and monitoring seed health in vegetatively propagated crops.

Advancements in seed health testing methods have revolutionized disease detection and management in agriculture. By employing a combination of traditional and molecular techniques, seed producers and researchers can effectively screen seed lots for pathogens, implement targeted disease control measures, and safeguard crop health and productivity.

Genetic Purity in Seeds

OECD, ISTA, and Indian Standards

The Significance of Genetic Purity

Maintaining seed genetic purity is fundamental for sustaining agricultural productivity and ensuring consistency in crop performance. Seeds encapsulate the genetic traits essential for desirable agricultural outcomes, including yield potential, disease resistance, and adaptation to specific environments. Genetic purity guarantees that these valuable traits are faithfully transmitted from one generation to the next, optimizing crop yields and supporting agricultural sustainability. Any deviation from genetic purity, whether due to unintentional mixing, genetic drift, or contamination with off-type plants, can compromise crop performance and erode farmers' confidence in the reliability of the seed supply system.

OECD Seed Schemes: Pre- and Post-Control Measures

The Organisation for Economic Co-operation and Development (OECD) Seed Schemes play a pivotal role in facilitating international seed trade by harmonizing standards and streamlining certification procedures. These schemes incorporate comprehensive measures for ensuring genetic purity, encompassing both "pre-control" and "post-control" mechanisms:

Pre-control:

- Variety Descriptions: Standardized descriptions ensure accurate identification and selection of breeder's stock, minimizing the risk of genetic impurities.
- Field Inspections: Regular inspections of seed multiplication fields detect any potential contamination and ensure compliance with purity standards.
- Seed Sampling and Testing: Rigorous analyses of seed samples for purity and quality provide assurance of genetic integrity and adherence to established standards.

Post-control:

- Trade Inspections: Imported seed lots are subjected to verification against certification documents and may undergo additional testing to confirm genetic purity.
- Grow-Out Tests: Samples from certified seed lots are planted under controlled conditions to visually assess genetic trueness in the resulting plants, providing a final check on genetic purity.

These meticulous measures safeguard against genetic contamination within and across borders, fostering trust and confidence in the international seed market.

ISTA Guidelines: Standardized Seed Testing Procedures

The International Seed Testing Association (ISTA) serves as the global authority on seed quality testing, establishing internationally recognized procedures for genetic purity determination. ISTA's guidelines encompass a range of methodologies, including:

- **Morphological and Biochemical Tests**: Seed characteristics such as shape, size, and color can be utilized to identify off-type individuals. Biochemical markers, such as isozymes, offer additional tools for distinguishing between varieties. These methods rely on observing physical characteristics and biochemical markers of seeds to identify off-type individuals. Morphological traits such as shape, size, and color can provide valuable insights into genetic purity. Additionally, biochemical tests, including the analysis of isozymes, offer supplementary tools for distinguishing between different varieties. By examining these traits, seed testers can assess the degree of conformity to established standards and identify any deviations indicative of impurities.
- Molecular Techniques: Advanced methods such as DNA fingerprinting provide highly accurate and sensitive means of assessing genetic purity. ISTA recommends the use of advanced molecular methods for precise genetic purity assessment. Techniques such as DNA fingerprinting leverage the unique genetic signatures inherent in each seed variety to provide highly accurate and sensitive means of detection. By analyzing the DNA profiles of seed samples, testers can effectively identify and differentiate between cultivars, ensuring compliance with purity standards. Molecular techniques offer unparalleled resolution and reliability, making them indispensable tools in modern seed testing practices.
- Grow-Out Tests: Similar to the OECD approach, ISTA recommends the use of grow-out tests under controlled conditions to visually assess genetic uniformity in a representative sample of plants.

Following standardized ISTA procedures ensures data comparability and enhances confidence in the reported genetic purity of seed lots.

Indian Minimum Seed Certification Standards: The Grow Out Test

India, being a major agricultural producer, has developed its own seed certification system through the Indian Minimum Seed Certification Standards (IMSCS). A notable component of this system is the Grow Out Test (GOT), which is mandatory for specific hybrid and notified varieties of various crops.

The GOT entails:

- Sample Collection: Representative seed samples are collected from certified seed lots. See working sample size in Annex 3
- Planting in test plots: Seeds are planted under controlled conditions alongside a standard control sample of the same variety.(see Annex 6 for Grow Out Test details on planting the test plots)
- Visual Assessment: Throughout the growth cycle, plants are carefully examined for any off-type characteristics deviating from the standard control.
- Determination: If the number of off-type plants exceeds the prescribed tolerance limit, the seed lot fails the test and cannot be certified for sale.

Ensuring seed genetic purity is a multifaceted endeavor that requires collaboration among international organizations, national authorities, and seed testing laboratories. OECD Seed Schemes, ISTA guidelines, and the Indian Minimum Seed Certification Standards, including the Grow Out Test, serve as key frameworks for harmonizing seed quality regulations and guaranteeing reliable seed performance for farmers worldwide. By continually refining testing methodologies and strengthening international cooperation, the global seed industry can further uphold the genetic integrity of seeds, facilitating agricultural advancements and securing food security for future generations.

Quality Control in Seed Testing

In the domain of seed testing, a multifaceted approach is essential to guarantee reproducibility, standardization, and accuracy across various stages of analysis. This is critical to ensure the health, viability, and quality of seeds, which are foundational to agriculture and food security. The measures listed below provide a comprehensive framework to achieve these objectives:

1. Standard Operating Procedures (SOPs)

Standard Operating Procedures are detailed, written instructions designed to achieve uniformity of the performance of a specific function. SOPs are crucial in seed testing for ensuring that all procedures—from sample reception to germination testing—are conducted consistently across analysts and laboratories. These documents serve as a reference guide, ensuring that every step of the process is executed according to best practices, thereby minimizing variability and enhancing reproducibility (International Seed Testing Association [ISTA], 2021).

2. Harmonized Standards

Harmonization refers to the adoption of universally accepted standards and protocols. In seed testing, ISTA prescribes a set of rules and standards that are globally recognized. These standards cover various aspects of seed testing, including sampling methodologies, test procedures, and reporting formats. The adoption of harmonized standards facilitates the comparability of seed testing results across different laboratories worldwide, promoting international trade and ensuring seed quality (ISTA, 2021).

3. Regular Personnel Training

Continuous and regular training programs for personnel are imperative to maintain a high level of competence in seed sample preparation, testing methodologies, evaluation techniques, and statistical analyses. Training ensures that personnel are updated on the latest methodologies, technologies, and quality control measures. It is also essential for new employees to achieve the required competency level and for all staff to maintain their skills over time (Association of Official Seed Analysts [AOSA], 2020).

4. Automated Equipment

The integration of automated equipment in seed testing laboratories enhances the speed and precision of analyses while reducing the potential for human error. Automated systems for counting, sorting, and analyzing seeds can significantly improve the efficiency and accuracy of seed testing procedures. Regular maintenance and calibration of these systems are crucial to ensure their optimal performance (ISTA, 2021).

5. Calibration and Checks

Regular calibration of equipment and periodic checks using reference standards and control samples are essential to verify the accuracy and reliability of testing equipment. This practice helps identify and correct deviations, ensuring that the equipment provides precise and accurate results consistently. Calibration and quality checks are fundamental components of a laboratory's quality assurance program (AOSA, 2020).

6. Labeling Systems

A robust labeling system is vital for tracking samples throughout the testing process. Labels should include essential details such as the analyst's name, date of analysis, replicate number, and equipment used. This system enhances traceability and accountability, facilitating the identification of samples and associated data at any stage of the testing process (ISTA, 2021).

7. Statistical Design and Tolerances

The application of statistical principles in the design of seed testing experiments, including appropriate replication, randomization, sample size determination, and the establishment of permitted variances, is critical for the reliability of test results. These principles help in minimizing bias and ensuring the statistical significance of the findings, thus enhancing the decision-making process regarding seed quality (ISTA, 2021).

8. Quality Assurance

Quality assurance encompasses a wide range of activities, including accreditation of laboratories, participation in external audits, the use of blind check samples, and the analysis of performance statistics. These activities are designed to monitor and maintain the quality of seed testing services, ensuring that laboratories adhere to international standards and produce reliable, accurate results (ISTA, 2021).

9. Digital Data Management

The implementation of an Integrated Laboratory Information Management System (LIMS) is crucial for the efficient storage, analysis, and reporting of seed testing data. A LIMS facilitates the automation of workflows, enhances data integrity, and supports compliance with quality standards. It also enables efficient data retrieval, analysis, and sharing, thereby improving the overall efficiency and reliability of the seed testing process (ISTA, 2021).

In conclusion, the implementation of these measures is paramount for maintaining the integrity, reliability, and standardization of seed testing processes. By adhering to these practices, laboratories can ensure the production of high-quality, reliable seed testing data, which is essential for supporting agriculture and food security globally.

References

- Association of Official Seed Analysts (AOSA). (2020). Seed Testing Standards.
- International Seed Testing Association (ISTA). (2021). International Rules for Seed Testing.

Documentations in a seed testing laboratory

Introduction

Documentation and document handling form the backbone of quality assurance in seed testing laboratories. This chapter delves into the intricacies of various documentation types, their significance, and systematic management to ensure the integrity and reliability of seed testing processes.

Types of Documentation

1. Operational Manual

The Operational Manual is a comprehensive document detailing the laboratory's operations, including its objectives, responsibilities, and regulatory compliance. It serves as a foundational guide for all laboratory activities, ensuring that operations are aligned with established standards and practices.

2. Work Instructions

Work Instructions are technical documents that provide detailed "how-to" guides for conducting specific tests. These documents are essential for standardizing testing procedures and ensuring consistency and accuracy in test results.

3. Forms and Data Recording

Forms and data recording mechanisms, including both hard copy registers and electronic data entry points, are critical for documenting test results and other relevant data. This dual approach facilitates data integrity and traceability.

Document Handling Procedures

Sample Processing Flow

The sample processing flow in a seed testing laboratory, as outlined in the provided document, involves a detailed and systematic approach to ensure the accuracy and reliability of test results. This process can be expanded into the following detailed steps:

1. Sample Reception

- Upon arrival, each seed sample is logged into a tracking system, typically using a unique identification code. A job card is filled up at this stage which moves with the working sample to all the testing benches
- The sample's physical condition, quantity, and accompanying documentation are verified against submission forms to ensure completeness and compliance with testing requirements.

2. Sample Division

- Samples are carefully divided into portions required for different tests. This division is crucial for maintaining the representativeness of each test portion.
- Proper division techniques are employed to minimize damage to seeds and ensure that the sample portions accurately reflect the original sample's composition.

3. Testing Phases

- Each portion undergoes specific tests as per the laboratory's protocols. Common tests include germination, physical purity, moisture content, and seed health.
- Standard Operating Procedures (SOPs) guide each test to ensure consistency and repeatability of results across different analysts and test runs.

4. Data Recording and Analysis

- Results from each test are meticulously recorded on designated forms or into a Laboratory Information Management System (LIMS). This includes recording observations, measurements, and any deviations from expected outcomes.
- Data is analyzed using statistical tools and looked up with the tolerance tables to determine the seed lot's quality and compliance with regulatory standards of tolerance.

5. Quality Control Checks

- Throughout the testing process, quality control samples are analyzed alongside test samples to ensure the testing equipment and methodologies are performing as expected.
- Any anomalies or deviations identified during quality control checks prompt a review of the testing process and, if necessary, retesting of samples.

6. Compilation of Results

- Once all tests are completed, results are compiled and reviewed for accuracy and completeness. This often involves cross-checking data entries and calculations.
- A final report is prepared, summarizing the findings from all tests conducted on the sample. This report includes interpretations of the results and recommendations based on the seed lot's quality.

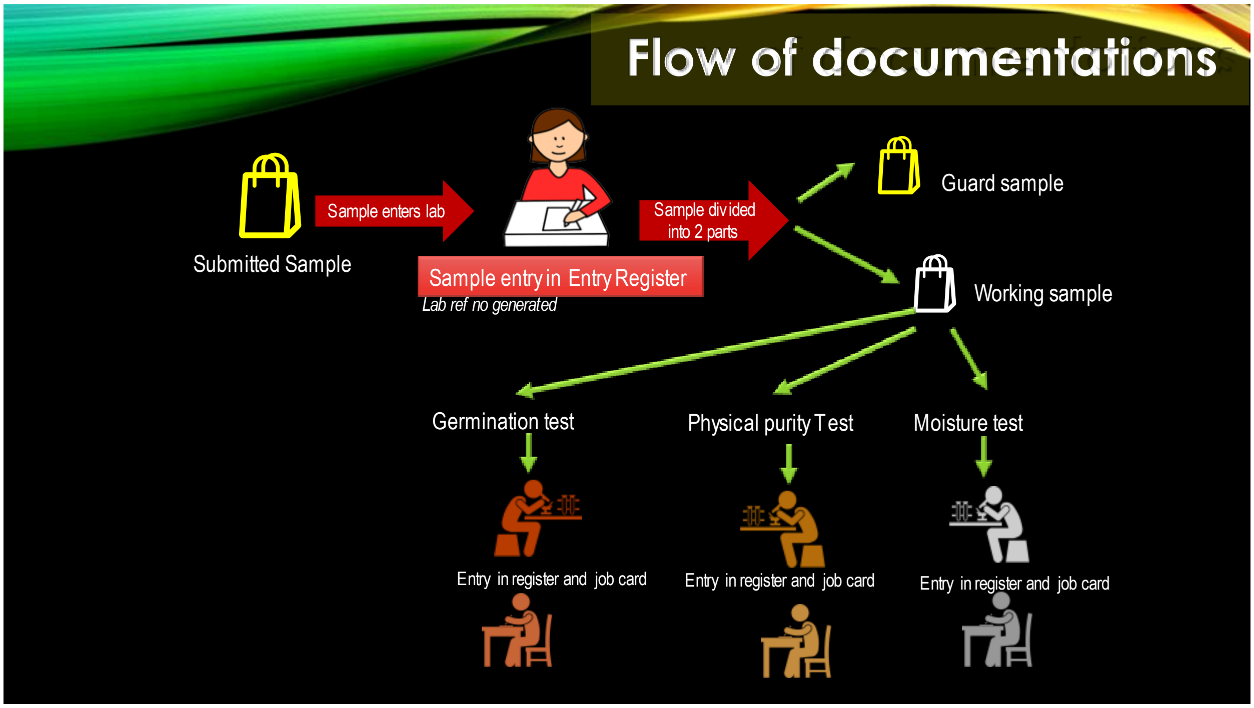
7. Result Reporting

- The final report is reviewed by a senior laboratory staff or quality assurance team before being released to the client.
- Care is taken to ensure the report is clear, accurate, and provides meaningful insights into the seed lot's quality and suitability for its intended use.

8. Sample Retention and Disposal

- After testing, samples are retained for a specified period to allow for any queries or additional tests that may arise from the initial results.
- Samples are disposed of following the laboratory's environmental and safety protocols once the retention period expires.

This detailed process ensures that each step, from sample reception to result reporting, is executed with precision and care, maintaining the integrity of the testing process and providing stakeholders with reliable and actionable information on seed quality.



Flow of documentations

Submitted Sample

Sample enters lab

Sample entry in Entry Register

Lab ref no generated

Sample divided into 2 parts

Guard sample

Working sample

Germination test

Physical purity Test

Moisture test

Entry in register and job card

Entry in register and job card

Entry in register and job card

Key Considerations

- **Accuracy and Clarity**: Documentation must be clear, accurate, and comprehensive to ensure that all personnel can follow procedures and protocols without ambiguity.
- **Regular Updates and Reviews**: Documents should be regularly reviewed and updated to reflect changes in regulations, standards, and laboratory practices.
- **Training and Competence**: Personnel should be trained in document handling procedures, emphasizing the importance of accurate documentation and data integrity.
- **Data Security and Confidentiality**: Electronic data management systems must be secure and comply with data protection regulations to protect sensitive information.
- **Quality Assurance**: Documentations and document handling practices should be integrated into the laboratory's quality assurance framework, with regular audits and checks to ensure compliance with standards.

Conclusion

Effective documentation and document handling are critical for maintaining the integrity, reliability, and standardization of seed testing processes. By adhering to rigorous documentation practices, seed testing laboratories can achieve high levels of quality assurance, facilitating the delivery of accurate and reliable test results.

Scope for Improvement in Seed Testing

While current practices and technology enable satisfactory performance of quality assurance functions, opportunities exist for enhancing seed testing speed, precision, sensitivity, informational value, integration and throughput. Potential advancements include:

1. Automated Smart Sampling: Real-time sensors for bulk seed lots to guide AI-driven targeted sampling and representativeness checks.
2. Hyperspectral Imaging: Detection of contaminants, defects and physiological state based on spectral signature analysis.
3. Computer Vision: Automated seedling image analysis for faster, more consistent vigor and germination evaluation.
4. Molecular Tools: DNA fingerprinting, qPCR, lateral flow strips and high-throughput sequencing for varietal ID, purity checks and disease diagnostics.
5. Spectral Phenomics: Chlorophyll fluorescence, NMR, FTIR and Raman spectroscopy for non-destructive seed quality profiling.
6. Wireless Sensors: Embedded sensor networks to monitor seed microclimate and metabolism aiding prediction of longevity and vigour.
7. Knowledge Systems: Comprehensive databases and expert systems to aid quality assessment and support decision making.
8. Blockchain Integration: Cryptographic traceability mechanisms to track seed movement, analyze trends and ensure transparency.

Advances in robotics, automation, remote sensing, information technology and genomics coupled with interdisciplinary collaboration can transform seed testing to become faster, more detailed and integrated with on-farm and on-line systems for smart seed quality management.

Seed testing provides the foundation for quality assurance, regulation and performance prediction in the seed supply chain. As the first link in the cycle of productivity, quality seed establishes the yield potential. Reliable seed testing safeguards farmers against risks from poor quality seed. Globally harmonized procedures, continued research on seed physiology and viability markers, automation, molecular diagnostics and IT integration can enhance the capability, information value and user access to seed testing systems. However, ensuring representative samples remains the most critical factor for seed quality testing accuracy. Integrating seed testing with certification, conditioning, storage and field monitoring can enable proactive management of seed quality factors from breeding through delivery to sustain crop productivity and food security.

References

AOSA (2022). Rules for testing seeds. Association of Official Seed Analysts. https://www.analyzeseeds.com/rules-for-testing-seeds

Elias, S.G., & Copeland, L.O. (1997). The US national seed quality system for soybean. Seed Science Research, 7(3), 255-264.

ISTA (2022). International rules for seed testing. International Seed Testing Association. https://www.seedtest.org/upload/cms/user/ISTA_Rules_2022_chapter4_sampling_MV.pdf

Lakon, G. (1949). The topographical tetrazolium method for determining the germinating capacity of seeds. Plant physiology, 24(3), 389.

More information

Additional Tests

Besides routine quality tests like purity, moisture, germination and vigor, several other laboratory and field-based tests provide valuable supplemental data:

1. Field Emergence and Survival: Planted samples monitored for emergence percentage, speed and seedling survival to predict performance.
2. Seed Aging Tests: Controlled deterioration to assess relative longevity and shelf-life potential.
3. Insect Damage Tests: X-ray imaging or embryo excision to enumerate internal feeding injuries.
4. Seed Weight: Hundred-seed weight indicates seed development and potential reserves.
5. Biochemical Tests: Enzyme activity, conductivity, respiration rate and metabolites as viability marker.
6. Pathology Tests: Grow-out, ELISA, DNA/RNA assays for specific pathogens.
7. Cytology and Imaging: Microscopy, radiography and tomography to visualize internal morphology and defects.
8. Ploidy Testing: Flow cytometry and chromosome counts to verify hybridity and purity.
9. DNA Fingerprinting: Molecular markers for varietal identity confirmation and parental verification.
10. Trait Verification: Grow-outs, chemical assays and instrumental analysis to validate special traits like oil, protein, pigments, and bioactives.

While not routine, targeted deployment of special tests provides additional useful information on seed characteristics to guide quality enhancement, conditioning, processing, storage, marketing and regulatory actions.

Automation in Seed Testing

Manual seed testing procedures are labor-intensive, slow and prone to subjective errors. Automation aims to accelerate sample throughput, improve precision and minimize drudgery. Current applications include:

1. Automatic Seed Counters: Precision dispensing of seed numbers for replicates using vibratory trays, vacuum pickups or robotics.
2. Programmable Incubators: Automated temperature, humidity, lighting and imaging systems for germination testing with data logging capabilities.
3. Seedling Evaluation Systems: Machine vision and image analysis tools for vigor and morphology assessments.
4. Near Infrared Reflectance (NIR) Sorting: Spectral detection of contaminants and foreign material for automatic separation.
5. Robotics: Robotic arms for sample transfer and handling to minimize human errors.
6. Laboratory Information Systems (LIS): Databases for test scheduling, processing, analysis and certificate generation.
7. Moisture Meters: Portable instant digital readout meters for rapid estimation.
8. Hyperspectral Imaging: Non-destructive seed quality evaluation based on spectral signature analysis.

Automation enhances the speed, precision and objectivity of seed testing besides improving working conditions for analysts. However, initial investments, technical skills and standardization efforts are needed for widespread adoption. Collaboration between engineers, data scientists and seed analysts will be key to develop practical integrated automation solutions.

In summary, standardized scientifically robust seed testing protocols, automation and quality assurance practices enable stakeholders to objectively evaluate seed lot value, make informed decisions and prevent introduction of poor quality planting material into the supply chain. Reliable seed testing thereby provides the foundation for realizing the benefits of quality seed - the vehicle to improve agricultural productivity, sustainability and food security across regions and farming systems.

Annex 1: Apparatus needed for seed testing

The apparatus required for seed testing can be categorized based on the type of test and the stage of sampling. The following is a list of the main apparatus for each category:

- **For primary sample collection** (for seed sampling from seed lot):
    - Bin trier/sampler
    - Sleeve or stick trier
    - Nobbe trier
- **For secondary sample preparation** (for submitted sample and working sample):
    - Seed dividers
        - Boerner type (conical)
        - Gamet type (centrifugal)
        - Riffle type (soil type)
    - Sample pans (plastic or aluminum) - assorted
    - Pan type weighing balance
    - Buckets (plastic) - assorted
    - Containers - bags
    - Stickers and labels
- **For purity analysis**:
    - Purity work board/table (diaphanoscope)
    - Spatula
    - Forceps
    - Brushes
    - Purity dishes
    - Magnifying lens - 5X, 6X, 10X
    - Set of sieves (for seeds)
    - Electrical balance - single pan (up to 1 kg) (up to 1 place of decimal)

  - Fluorescent lamps
  - UV lamp
  - Stereoscopic binocular microscope
  - Seed blower
  - Seed herbarium
  - Sample trays
  - Electrophoretic apparatus
- **For viability test** of a seed sample:
  - Staining dishes - watch glass
  - Blades, needles, forceps
  - Dispensing bottles, droppers
  - B.O.D
  - Magnifying glass
- **For germination test and vigour test**:
  - Glass plates (10 x 15 cm and 15 x 20 cm)
  - Plastic covers
  - Blotting paper roll
  - Rubber bands
  - Tray holder
  - B.O.D
  - Refrigerator
  - Thermometer
  - Petri dishes
  - Plastic buckets
  - Plastic tray - surgical
  - Forceps
  - Counting board
  - Germination paper
  - Seed germinator

  - Seed scarifier
  - Desiccators
  - Silica gel
  - Soil and sand boxes
  - Vacuum counter
  - Soil and sand boxes and sterilizers
  - Sprinklers
  - Magnifying glass
  - Sample trays
- **For moisture estimation** of a seed sample:
  - Hot air oven
  - Sample weighing container: aluminum box with lids and clamp or glass bottle
  - Seed grinder
  - Desiccator
  - Tongs
  - Silica gel
  - Moisture meter
  - Sample pan
  - Analytical balance - electrical
  - Sieves
- **For seed health test**:
  - Stereoscopic microscope
  - Autoclave
  - Oven
  - Incubator
  - B.O.D
  - General glass wares and other commonly used equipment for plant pathology laboratory
- **For tolerance test**:

    - Calculator
- **Other equipment**:
    - Growth chamber
    - Respirometer
    - Lab model seed cleaning machine, e.g. air screen machine, spiral separator, disc separator, gravity separator, and aspirator
    - Hygrometer (for measuring humidity in the room temperature)

Annex 2: Maximum lot sizes

Crop Name	***Botanical Name***	**Maximum Lot Size (tons); 1 ton =1000 kg**
Chow-chow	*Sechium edule*	**40 tons**
Garlic	*Allium sativum*	
Lesser yam	*Dioscorea esculenta*	
Maize	*Zea mays*	
Multiplier onion	*Allium cepa var. aggregatum*	
Seed potato	*Solanum tuberosum*	
Sweet Potato	*Ipomoea batatas*	
Ashgourd (Petha)	*Benincasa hispida*	**20 tons**
Asparagus	*Asparagus officinalis*	
Barley	*Hordeum vulgare*	
Birdwood grass	*Cenchrus setigerus*	
Bitter gourd	*Momordica charantia*	
Black gram (Urdbean)	*Vigna mungo*	
Bottle gourd	*Lagenaria siceraria*	
Castor	*Ricinus communis*	
Chikling vetch (Khesari)	*Lathyrus sativus*	
Cluster bean (Guar)	*Cyamopsis tetragonoloba*	
Cotton	*Gossypium spp.*	
Cowpea (Asparagus bean)	*Vigna unguiculata*	
Garden beet	*Beta vulgaris*	
Gram (Bengal gram)	*Cicer arietinum*	
Green gram (Mung bean)	*Vigna radiata*	
Groundnut	*Arachis hypogaea*	
Horse gram (Kulthi)	*Macrotyloma uniflorum*	
Indian bean (Sem)	*Lablab purpureus*	
Indian squash (Tinda)	*Praecitrullus fistulosus*	
Moth bean (Kidney bean)	*Vigna aconitifolia*	
Oats	*Avena sativa*	
Okra (Bhindi)	*Abelmoschus esculentus*	
Paddy	*Oryza sativa*	

Pea	*Pisum sativum*	
Pigeon pea (Arhar)	*Cajanus cajan*	
Rajmash (French bean)	*Phaseolus vulgaris*	
Ridge gourd	*Luffa acutangula*	
Snake gourd	*Trichosanthes cucumerina*	
Soybean	*Glycine max*	
Spinach beet	*Beta vulgaris subsp. cicla*	
Sponse gourd	*Luffa aegyptiaca*	
Sugar beet	*Beta vulgaris subsp. vulgaris*	
Summer squash	*Cucurbita pepo var. cylindrica*	
Sunflower	*Helianthus annuus*	
Teosinte	*Zea mays subsp. mexicana*	
Triticale	× *Triticosecale*	
Watermelon	*Citrullus lanatus*	
Wheat	*Triticum aestivum*	
Winter squash	*Cucurbita maxima*	
Alfa alfa (Lucerne)	*Medicago sativa*	**10 tons**
Amaranth	*Amaranthus spp.*	
Barnyard millet (Sawan)	*Echinochloa frumentaceae*	
Berseem (Egyptian clover)	*Trifolium alexandrinum*	
Brinjal (eggplant)	*Solanum melongena*	
Broccoli	*Brassica oleracea var. italica*	
Buffel grass	*Cenchrus ciliaris*	
Cabbage	*Brassica oleracea var. capitata*	
Carrot	*Daucus carota subsp. sativus*	
Cauliflower	*Brassica oleracea var. botrytis*	
Celeriac	*Apium graveolens var. rapaceum*	
Celery	*Apium graveolens*	
Chinese cabbage	*Brassica rapa subsp. pekinensis and Brassica rapa subsp. chinensis*	
Common millet (Cheema)	*Panicum miliaceum*	
Cucumber	*Cucumis sativus*	
Dharaf grass	*Cenchrus ciliaris*	
Dinanath grass	*Pennisetum pedicellatum*	
Fenugreek (methi)	*Trigonella foenum-graecum*	
Finger millet (Ragi)	*Eleusine coracana*	
Guinea grass	*Panicum maximum*	
Hot pepper (chilli)	*Capsicum annuum*	
Italian millet (Kangni)	*Setaria italica*	
Jute	*Corchorus spp.*	
Knol-kohl	*Brassica oleracea var.gongylodes*	
Kodo millet (Kodo)	*Paspalum scrobiculatum*	
Lentil	*Lens culinaris*	
Lettuce	*Lactuca sativa*	

Linseed	*Linum usitatissimum*
Little millet (Kutki, samai)	*Panicum sumatrense*
Longmelon (Kakri)	*Cucumis melo var. utilissimus*
Marvel grass	*Dichanthium annulatum*
Muskmelon	*Cucumis melo*
Niger (Ramtil)	*Guizotia abyssinica*
Onion	*Allium cepa*
Parsley	*Petroselinum crispum*
Pearlmillet (Bajra)	*Pennisetum glaucum*
Pumpkin	*Cucurbita pepo*
Radish	*Raphanus sativus*
Rapeseed & Mustard	*Brassica spp.*
Rat-tail radish (Mungra)	*Raphanus caudatus*
Rice bean	*Vigna umbellata*
Rocket Salad (Taramira)	*Eruca sativa*
Safflower (Kardi, Kusum)	*Carthamus tinctorius*
Sesame (Til)	*Sesamum indicum*
Setaria grass	*Setaria italica*
Snapmelon (Phoont)	*Cucumis melo var. momordica*
Sorghum	*Sorghum bicolor*
Spinach	*Spinacia oleracea*
Stylo	*Stylosanthes spp.*
Sweet clover (Senji)	*Melilotus spp.*
Sweet pepper	*Capsicum annuum*
Tomato	*Solanum lycopersicum*
True potato seed (TPS)	*Solanum tuberosum*
Turnip	*Brassica rapa subsp. rapa*

Anexe 3: Working samples minimum weight required for Physical Purity and Genetic Purity

Category	Crop	Botanical Name	Minimum Working Sample Wt. (gm) - Physical Purity	Minimum Working Sample Wt. (gm) - Genetic Purity/ Grow Out Test
CEREALS	Barnyard millet	*Echinochloa crus-galli*	8	80
	Common millet	*Panicum miliaceum*	15	150
	Italian millet	*Setaria italica*	9	90
	Barley	*Hordeum vulgare*	120	1000
	Maize	*Zea mays*	900	1000
	Paddy/ Rice	*Oryza sativa*	40	400
	Pearl millet	*Pennisetum glaucum*	15	150
	Oats	*Avena sativa*	120	1000
	Sorghum	*Sorghum bicolor*	90	900
	Wheat	*Triticum aestivum*	900	1000
PULSES	Black gram	*Vigna mungo*	700	1000
	Chick pea	*Cicer arietinum*	1000	1000
	Cowpea	*Vigna unguiculata*	400	1000

	Field bean	*Vicia faba*	500	1000
	French bean	*Phaseolus vulgaris*	700	1000
	Green gram	*Vigna radiata*	120	1000
	Lentil	*Lens culinaris*	60	600
	Lablab bean	*Lablab purpureus*	500	1000
	Lima bean	*Phaseolus lunatus*	1000	1000
	Pea	*Pisum sativum*	900	1000
	Pigeon pea	*Cajanus cajan*	300	1000
	Soybean	*Glycine max*	500	1000
OILS & FATS	Castor	*Ricinus communis*	500	1000
	Groundnut	*Arachis hypogaea*	1000	1000
	Safflower	*Carthamus tinctorius*	90	900
	Sesame	*Sesamum indicum*	7	70
	Sunflower	*Helianthus annuus*	200	1000
	Rape & Mustard	*Brassica rapa & Brassica juncea*	4	40
	Linseed	*Linum usitatissimum*	15	150
SUGAR & STARCHES	Buckwheat	*Fagopyrum esculentum*	60	600
	Sugar beet	*Beta vulgaris subsp. vulgaris*	50	500
FORAGE CROPS	Berseem	*Trifolium alexandrinum*	6	60
	Blue panic	*Panicum antidotale*	2	20
	Buffelo grass	*Cenchrus ciliaris*	2	20
	Canary grass	*Phalaris aquatica*	4	40
	Carpet grass	*Axonopus affinis*	1	10
	Clovers	*Trifolium spp.*	0.5-25	5 to 250
	Dallies grass	*Paspalum dilatatum*	5	50
	Guinea grass	*Megathyrsus maximus*	2	20
	Lucerne	*Medicago sativa*	5	50
	Lupines	*Lupinus spp.*	450	1000
	Napier grass	*Pennisetum purpureum*	5	50
	Orchard grass	*Dactylis glomerata*	3	30
	Tall fescue	*Festuca arundinacea*	5	50
	Vetches	*Vicia spp.*	120-1000	1000
	Weeping love grass	*Eragrostis curvula*	1	10
GREEN MANURES	Cluster beans	*Cyamopsis tetragonoloba*	80	800
	Sunnhemp	*Crotalaria juncea*	70	700
BEVERAGES	Chicory	*Cichorium intybus*	5	50
	Tobacco	*Nicotiana tabacum*	0.5	5
LEGUME VEGETABLES	Broad bean	*Vicia faba*	1000	1000

	Cluster bean	*Phaseolus vulgaris*	80	800
	Cowpea	*Vigna unguiculata*	400	1000
	Dolichos bean	*Dolichos lablab*	500	1000
	French bean	*Lablab purpureus*	700	1000
	Garden pea	*Pisum sativum*	900	1000
	Lima bean	*Phaseolus lunatus*	1000	1000
	Scarlet runner bean	*Phaseolus coccineus*	1000	1000
CUCURBITS	Long melon	*Cucumis melo var. flexuosus*	70	150
	Cucumber	*Cucumis sativus*	70	150
	Indian squash	*Cucurbita moschata*	250	1000
	Muskmelon	*Cucumis melo var. cantalupensis*	70	150
	Pumpkin	*Cucurbita pepo*	180	350
	Summer squash	*Cucurbita pepo subsp. pepo*	180	350
	Water melon	*Citrullus lanatus*	250	1000
	Winter squash	*Cucurbita maxima*	700	1000
LEAFY VEGETABLES	Celery	*Apium graveolens var. dulce*	1	10
	Lettuce	*Lactuca sativa*	3	30
	New Zealand spinach	*Tetragonia tetragonoides*	200	1000
	Spinach	*Spinacia oleracea*	25	250
ROOT VEGETABLE	Beet	*Beta vulgaris subsp. vulgaris*	50	500
	Carrot	*Daucus carota subsp. sativus*	3	30
	Parsnip	*Pastinaca sativa*	10	100
	Radish	*Raphanus sativus*	30	300
	Turnip	*Brassica rapa subsp. rapa*	7	70
BULBS	Leek	*Allium ampeloprasum*	7	70
	Onion	*Allium cepa*	8	80

Annex 4: Tolerances and tolerance tables

A tolerance level of a seed test is the statistically permissible level of variation in the results at a given probability level. If the test results are within the permissible level of tolerance, it indicates that the difference between the replicates or between the two independent tests or otherwise is non-significant and hence tests conducted are good and acceptable.

Tolerances for comparing duplicate working samples from the same submitted sample for any component of physical purity for chaffy or non-chaffy seeds (ISTA Table)

The probability is 0.05

Average analysis of two half samples or two whole samples		**Tolerance for difference between**	
1	**2**	**Half working samples 3**	**Whole working samples 4**
99.95- 100	0.00-0.04	0.23	0.16
99.90-99.94	0.05-0.09	0.34	0.24
99.85-99.89	0.10-0.14	0.42	0.30
99.80-99.84	0.15-0.19	0.49	0.35
99.75-99.79	0.20-0.24	0.55	0.39
99.70-99.74	0.25-0.29	0.59	0.42
99.65-99.69	0.30-0.34	0.65	0.46
99.60-99.64	0.35-0.39	0.69	0.49
99.55-99.59	0.40-0.44	0.74	0.52
99.50-99.54	0.45-0.49	0.76	0.54
99.40-99.49	0.50-0.59	0.82	0.58
99.30-99.39	0.60-0.69	0.89	0.63
99.20-98.29	0.70-0.79	0.95	0.67
99.10-99.19	0.80-0.89	1.00	0.71
99.00-99.09	0.90-0.99	1.06	0.75
98.75-98.99	1.00-1.24	1.15	0.81
98.50-98.74	1.25-1.49	1.26	0.89
98.25-98.49	1.50-1.74	1.37	0.97
98.00-98.24	1.75-1.99	1.47	1.04

97.75-97.99	2.00-2.24	1.54	1.09
97.50-97.74	2.25-2.49	1.63	1.15
97.25-97.49	2.50-2.74	1.70	1.20
97.00-97.24	2.75-2.79	1.78	1.26
96.50-96.99	3.00-3.49	1.88	1.33
96.00-96.49	3.50-3.99	1.99	1..41
95.50-95.99	4.00-4.49	2.12	1.50
95.00-95.49	4.50-4.99	2.22	1.57
94.00-94.99	5.00-5.99	2.38	1.68
93.00-93.99	6.00-6.99	2.56	1.81
92.00-92.99	7.00-7.99	2.73	1.93
91.00-91.99	8.00-8.99	2.90	2.05
90.00-90.99	9.00-9.99	3.04	2.15
88.00-89.99	10.00-11.99	3.25	2.30
86.00-87.99	12.00-13.99	3.49	2.47
84.00-85.99	14.00-15.99	3.70	2.62
82.00-83.99	16.00-17.99	3.90	2.76
80.00-81.99	18.00-19.99	4.07	2.88
78.00-79.99	20.00-21.99	4.23	2.99
76.00-77.99	22.00-23.99	4.37	3.09
74.00-75.99	24.00-25.99	4.50	3.18
72.00-73.99	26.00-27.99	4.61	3.26
70.00-71.99	28.00-29.99	4.71	3.33
65.00-69.99	30.00-34.99	4.86	3.44
60.00-64.99	35.00-39.99	5.02	3.55
50.00-59.99	40.00-49.99	5.16	3.65

Maximum tolerated ranges in germination percent for deciding whether to retest; allowing for random sampling variation only (ISTA Table)

Average percent germination		**Number of replicates of 100 seeds**		
1	**2**	**3** **4 replications**	**4** **3 replications**	**5** **2 replications**
99	2	5	4	---
98	3	6	5	---
97	4	7	6	5
96	5	8	7	6
95	6	9	8	7
93-94	7-8	10	9	8
91-92	9-10	11	10	9
89-90	11-12	12	11	10
87-88	13-14	13	12	11

84-86	15-17	14	13	12
81-83	18-20	15	14	12
78-80	21-23	16	15	13
77	24	17	15	13
73-76	25-28	17	16	14
71-72	29-30	18	16	14
67-70	31-34	18	17	15
64-66	35-37	19	17	15
56-63	38-45	19	18	15
51-55	46-50	20	18	14

Tolerance for deciding whether germination test conducted on same sample is compatible; allowing for random sampling variation only

(ISTA table)

Average percent germination		**Tolerance**
1	**2**	**3**
98-99	2-3	2
95-97	4-6	3
91-94	7-10	4
85-90	11-16	5
77-84	17-24	6
60-76	25-41	7
51-59	42-49	8

Tolerances for comparing the laboratory result of germination test with a specified minimum limit of germination standard

Specified percent germination		400 seeds	800 seeds	200 seeds
1	**2**	**3**	**4**	**5**
99	2	1	1	2
98	3	2	1	3
97	4	2	1	3
96	5	2	2	4
95	6	3	2	4
94	7	3	2	4
93	8	3	2	5
92	9	3	2	5
91	10	4	2	5
90	11	4	3	6
89	12	4	3	6
88	13	4	3	6
87	14	4	3	6
86	15	5	3	7
1	**2**	**3**	**4**	**5**
85	16	5	3	7

84	17	5	3	7
83	18	5	3	7
82	19	5	4	7
81	20	5	4	8
80	21	5	4	8
79	22	6	4	8
78	23	6	4	8
77	24	6	4	8
76	25	6	4	8
75	26	6	4	9
74	27	6	4	9
73	28	6	4	9
72	29	6	4	9
71	30	6	4	9
70	31	7	5	9
69	32	7	5	10
68	33	7	5	10
67	34	7	5	10
1	**2**	**3**	**4**	**5**
66	35	7	5	10
65	36	7	5	10
64	37	7	5	10
63	38	7	5	10
62	39	7	5	10
61	40	7	5	10
60	41	7	5	10
59	42	7	5	10
58	43	7	5	10
57	44	8	5	11
56	45	8	5	11
55	46	8	5	11
54	47	8	5	11
53	48	8	5	11
52	49	8	5	11
51	50	8	5	11

For germination the above is used.

1. Lets say lab germination result is 85 and standard prescribed is 82
2. Calculate the difference which is 3
3. Considering the number of replicates as four out of 100 in each replicates the total number of seeds is 400
4. Fit the standard prescribed that is 85% in Table column 3
5. Tolerance label indicated is 6
6. Difference is 3 (step ii) which is less than 6 (the tolerance limit)
7. Conclusion: The laboratory result is in conformity with seed standards and seed lot can be sold as standard seed.

Annex 5: Standard germination testing procedures (ISTA)

B.P.: Between paper T.P.: Top of paper S.: Sand

20-30 means alternating temperature while 20,30 or 25, 30 means constant temperatures of that particular temperatures. A **dash “-”** implies alternating temperature while a **comma “,”** implies that that species can be germinated in a constant temperature of both those temperature regimes

Crop (Botanical Name)	Substrate	Temperature (°C)	First Count (days)	Final Count (days)	Additional Treatments
Cereals and Millets					
Barnyard millet (Echinochloa calona)	T.P., B.P.	20-30	4	7	Pre-drying at 40°C
Little millet (Panicum sumatrense)	T.P., B.P.	20-30	3	7	-
Maize (Zea mays)	B.P., S.	20-30	4	7	-
Paddy (Oryza sativa)	T.P., B.P., S	20-30	5	14	Pre-dry (40°C), GA$_3$
Pearl Millet (Pennisetum americanum); (P. typhoides)	B.P.	25-30	3	7	-
Finger millet (Elucine coracana)	T.P., B.P.	20-30	5	8	Pre-dry (40°C), GA$_3$
Sorghum (Sorghum bicolor)	B.P., S	20-30	4	10	Pre-dry (40°C), GA$_3$
Jowar (Sorghum vulgare)	T.P., B.P.	20-30	4	10	-do-
Pulses					
Black gram (Vigna mungo)	B.P., S	20-30	5	8	*Hot water treatment (80°C) for 1-2 minutes
Chickling Vetch (Khesari) (Lathyrus sativus)	B.P., S	20-30, 25	5	8	*Hot water treatment (80°C), for 1-2 minutes
Chick pea (Cicer arietinum)	B.P., S.	20	5	14	*Hot water treatment (80°C), for 1 minute

Cowpea (Vigna unguiculata)	B.P., S	20-30	5	8	*Hot water treatment (80°C), for 1-2 minutes
Green gram (Vigna radiata)	B.P.,S	20-30	3	7	*Hot water treatment (80°C) for 1-2 minutes
Horse gram (Dolichos biflorus)	B.P, S	20-30	5	10	*Hot water treatment (80°C), for 1 minute
Indian bean (Lablab Purpureus)	B.P, S	25	3	7	Do
Lentil (Lens esculenta), (L.culinaris)	B.P, S	20	4	10	*Hot water treatment (80°C), for 1 minute
Mothbean (Vigna aconitifolia)	B.P, S	20, 30	5	10	*Hot water treatment (80°C), for 1 minute
Pigeon pea (Cajanus cajan)	B.P, S	20-30	4	10	* Hot water treatment (80°C), for 1 minute
Faba bean (Vicia faba)	B.P, S	25, 30	5	8	Pre-chilling Scarification with sandpaper Conc. H_2SO_4 Treatment for 60 and 120 seconds
Rice bean (Vigna umbellata)	B.P.	20-30	5	8	Scarification with sandpaper Conc. H_2SO_4 Treatment for 60 and 120 seconds
Oilseeds					
Castor (Ricinus communis)	B.P., S	20-30	7	14	Predry (40°C)
Groundnut (Arachis hypogaea)	B.P.	20-30	5	10	-
Linseed (Linum usitatissimum)	T.P., B.P.	20-30	3	7	KNO_3
Mustard and rape (Brassica campestris, B. Juncea)	T.P.	20-30	4	7	Predry (40°C)
Safflower (Carthamus tinctorius)	B.P., S	20-30	3	6	Predry (40°C)
Seasame (Sesamum indicum)	T.P.	20-30	4	10	Predry (40°C)
Sunflower (Helianthus annus)	B.P., S	20-30	7	14	-
Fibre Crops					
Cotton (Gossypium species)	B.P., S	20-30	5	10	-
Jute (Corchorus capsularis), (Corchorus olitorius)	T.P; B.P	30	4	8	Light
Forage Crops					
Birdwood grass (Cenchrus setigerus)	T.P.; B.P	30	3	6	Low moisture
Cluster bean (Cyamopsis tetragonoloba)	B.P.	25	5	14	*Hot water treatment (80°C) for 1-2 minutes
Methi/ fenugreek	T.P.	25-30	4	8	Pre-dry (40°C)
Forage sorghum (Sorghum sudanense)	B.P.	20-30	3	7	*Hot water treatment (80°C) for 1-2 minutes
Marvel grass (Dichanthium annulatum)	B.P.	20-30	4	14	Pre-dry(40°C)
Setaria grass (Setaria anceps)	B.P.,S.	20-30	4	14	Pre-dry (40°C)
Stylo (Stylosathes spp.)	T.P	20-30	4	12	KNO_3
Medicinal Plants					
Isabgol (Plantago sps.)	B.P., S.	20-30	4	8	*Hot water treatment (80°C) for 5-10 minutes
Vegetable and Root Crops					
Amaranth (chauli) (Amaranthus tricolor)	T.P.	30	4	8	GA_3

Ash gourd (Petha) (Benincasa cerifera)	B.P.	20-30	5	14	*Low moisture
Asparagus (Asparagus-officinalis)	T.P.,B.P.,S.	20-30	7	21	*Low moisture
Bitter gourd (Momordica charantia)	B.P.,S.	25-30	3	7	GA_3
Bottle gourd (Lagenaria siceraria)	B.P.,S.	20-30	5	14	*Low moisture
Brinjal (Solanum melongena)	T.P.	30	4	8	GA_3
Cabbage (B. Oleracea var. capitata)	B.P.	20-30	5	14	GA_3
Capsicum (Capsicum annum)	T.P.,B.P.	20-30	7	21	*Low moisture
Carrot (Daucus carota)	T.P.,B.P	20-30	4	10	GA_3
Cauliflower (B.oleracea var. botrytis)	B.P.	25	3	7	-
Celery (Apium graveolens)	T.P.	20,25	4	8	* Low moisture
Chinese cabbage (B. pekinensis)	25-30	4	8	* Low moisture	
Cucumber (Cucumis sativus)	B.P.,S.	20-30	7	14	GA_3
Knolkhol (B. oleracea var. gongylodes)	T.P.	20,25	3	7	-
Okra (Abelmoschus esculentus)	B.P., S.	25,30	5	10	GA_3
Pointed gourd (Trichosanthes dioica)	B.P., S.	20,30	4	8	* Low moisture
Pumpkin (Cucurbita moschata)	B.P., S.	25,30	5	10	* Low moisture
Radish (Raphanus sativus)	B.P., S.	25,30	5	10	* Low moisture
Rat tail Radish (Raphanus caudatus)	B.P., S.	25,30	4	8	* Low moisture
Ridge gourd (Luffa cylindrica) and Snake gourd (Trichosanthes anguina)	T.P.	20-30	5	14	
Snap melon (Cucumis melo var momordica)	T.P.	20-30	3	7	
Sponge gourd (Luffa cylindrical)	B.P., S.	20-30	5	14	
Summer squash (Cucurbita pepo)					
Tomato (Lycopersicum esculentum) Turnip (Brassica rapa) Water melon (Citrullus lanatus) Winter squash (Cucurbita maxima)	B.P., S.	20-30	4	8	

Annex 6: Grow Out Test

(As per the Indian Standards. The below is taken from the Indian Minimum Seed Certification Standards 2019)

Grow-Out Test for Cultivar Purity

I. Object

The primary objective of the Grow-Out Test (GOT) is to ascertain the genetic purity of a given seed lot of a released cultivar. This test aims to evaluate the extent to which the submitted sample conforms to the prescribed standards, ensuring the integrity and reliability of the seed supply system.

II. Sampling

The samples for the grow-out test are to be drawn simultaneously with samples for other quality tests, following standard procedures. The size of the submitted sample varies according to the crop type:

- 1,000 gm: Maize, cotton, groundnut, soybean, and species of similar seed size.
- 500 gm: Sorghum, wheat, paddy, and species of similar seed size.
- 250 gm: Beta and species of similar seed size.
- 100 gm: Bajra, jute, and species of all other genera.
- 250 tubers/planting stakes/roots/corns: Seed potato, sweet potato, and other vegetatively propagating crops.

III. Procedure

The procedure involves raising the desired population under standard agronomic and cultural practices, ensuring uniformity between the unknown sample and its control. The test relies on the hereditary characteristics of plants, with cultivar differences being more distinct under favorable growth conditions. The examination entails mutual comparison between the samples to be tested and the standard sample, with standard samples sown at suitable intervals. Crop-specific specifications, including plot size and row length, are detailed in the provided table.

S. No.	Crop	Row Length (meters)	Plant to Plant Distance (cm)	Space Between Rows (cm)	Space Between Plots (cm)	No. of Replications
1	Wheat, barley, oats	6	2	25	50	2
2	Pea, cowpea	6	10	45	90	2
3	Chickpea, green gram, black gram	6	10	30	60	2
4	Maize	10	25	60	90	2
5	Hybrid cotton	5	10	45	45	2
6	Paddy / Rice	6	15	20	45	2

	Paddy (b)	6	25	30	60	2
7	Pearlmillet	6	10	60	90	2
8	Sorghum	6	10	45	60	2

IV. Observations

All plants are meticulously studied for distinguishing characters described for the cultivar, both in the test crop and the control. Observations are made throughout the growing period, with deviations from the standard sample recorded at suitable development stages. Plants exhibiting characteristics of other cultivars are identified and recorded accordingly.

V. Calculation, Interpretation, and Reporting of Results

The percentage of off-types is calculated to the first decimal place. Results are interpreted using tolerance tables provided, allowing for the application of tolerance when assessing conformity to prescribed standards.