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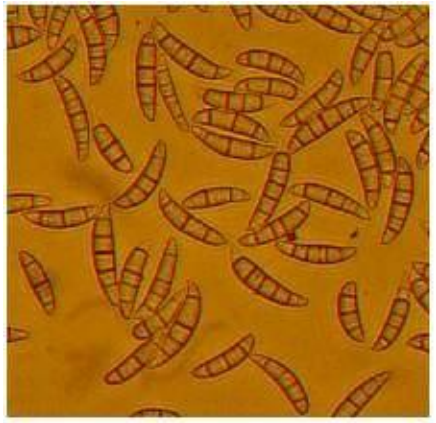
SEED ENTERPRISE MANAGEMENT INSTITUTE (SEMIs)

Seed Quality Assurance, Management and Control Processes

Seed Sampling Procedures



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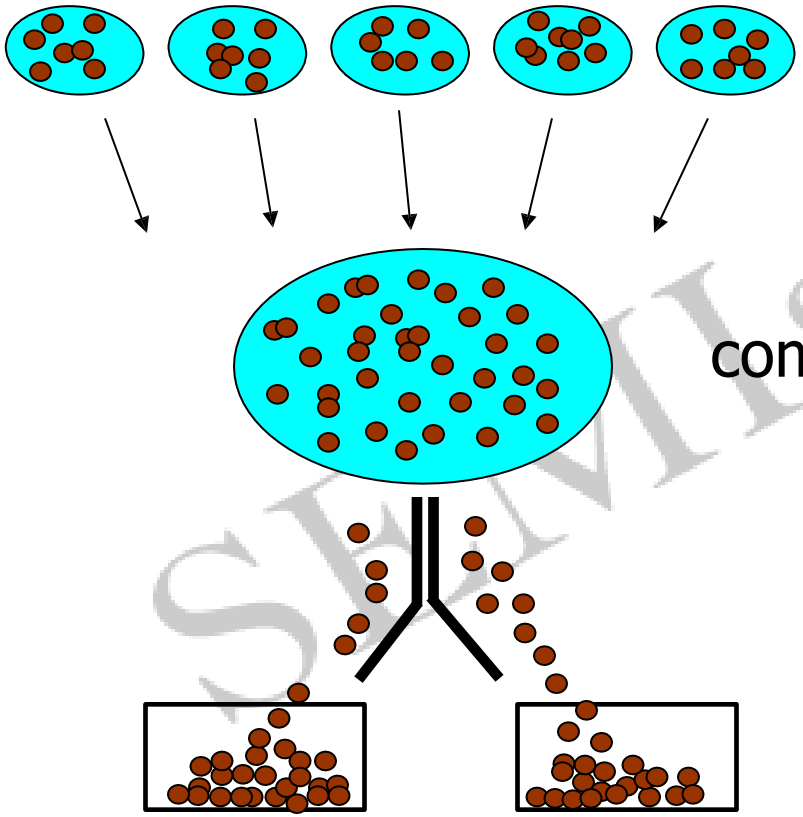
Sampling Definitions

- Seed lot - specified quantity of seed that is physically and uniquely identifiable (ISTA). A definite quantity of seed identified by a lot number or other mark, every portion or bag of which is uniform within recognized tolerances for the factors which appear in the labeling (AASCO).
- Primary sample - portion taken from the seed lot during a single sampling action.
- Composite sample - Formed by combining and mixing all primary samples.

Seed Sampling Procedures

- Sub-sample- portion of a sample obtained by reducing (dividing) a sample.
- Duplicate sample - another sample obtained for submission from the same composite sample and marked “Duplicate sample”.
- Working sample - the whole of the submitted sample or a sub-sample thereof, on which a quality test is made and must be at least the weight prescribed by ISTA for the particular test.

Samples - primary, composite, submitted, working



- Composite – combine all primary samples.
- Mix and sub-divide to get submitted (secondary) sample.
- Submitted sample may be further subdivided - working sample.

Considerations in seed sampling

- Seed lot to be sampled has to be uniform and representative in terms of composition and size of containers and has to be arranged in such a way in order for the sampler to have access to each and every container
- The sample itself is well mixed in the lab before portions are examined for purity, germination, and other testing.

Seed Sampling Procedures

- Heavy seeds tend to settle to the bottom of the sample bag, while lighter seeds tend to be at the top. This can be dealt with by passing seed through a laboratory divider or mixing the seed by hand, whether a purity analysis was requested or not.

Seed Sampling Procedures

- The seed lot shall not exceed the quantity indicated in the ISTA Rules, subject to a tolerance of 5%. Consignments that exceed these maximum sizes shall be sub-divided into separate, identifiable seed lots which do not exceed the maximum seed lot size.
- Sampling intensity is based on size of the seed lot and it refers to the minimum number of bags or containers of seed that should be sampled from a specific seed lot.

Seed Sampling Procedures

For seed lots in bags or containers that are of uniform size and are 15-100kg, the following is the minimum requirement:

- 1-4 containers, take 3 primary samples from each container.
- 5-8 containers, take 2 primary samples from each container.
- 9-15 containers, take 1 primary sample from each container.

Seed Sampling Procedures

- 16-30 containers, take 15 primary samples in total from the seed lot.
- 31-59 containers, take 20 primary samples in total from the seed lot
- 60 or more containers, take 30 primary samples in total from the seed lot

Seed Sampling Procedures

- For seed lots in containers smaller than 15kg , containers shall be combined into sampling units not exceeding 100kg, e.g. 20 containers of 5kg, 33 containers of 3kg or 100 containers of 1kg.
- For sampling purposes, each unit is regarded as one container and the sampling intensity prescribes for big containers will apply

Sampling methods

Non-mechanical methods

i) Spoon method - Used for obtaining seed for seed health testing; otherwise restricted to species smaller than wheat

- Mix seed and pour evenly over tray. Using spoon and spatula, remove small portions of seeds from not less than five random places.
- Take sufficient seed to constitute a sub-sample of required size.

ii) Modified halving method

- A grid of equal-sized cells, open at the top and alternate cells with no bottom, set on a tray.
- Seeds are mixed and poured evenly over grid. Grid is lifted and about half the sample remains on the tray.
- Sample is successively halved until sufficient quantities of seeds are obtained.

Mechanical dividers

i) Conical (Boerner) - Not motorized. Suitable for most kinds of seed (including chaffy); Not suitable for "stemmy" samples.

ii) Soil (Riffle): Not motorized. Better suited than Boerner for grasses and other species that may arrive to the Lab as a mass of florets and plant parts.

iii) Centrifugal (Gamet): Motorized. Especially useful for chaffy seed, but may clog due to a smaller throat than the Riffle divider.

iv) Rotary Divider

v) Variable Sample Divider

Seed Sampling Procedures

Conical

Riffle

Centrifugal

Riffle

Procedure for sampling

- Primary samples must be of approximately equal size regardless of the method
- When seed lot is in containers or bags, the bags to be sampled shall be selected at random throughout the seed lot and the primary samples shall be drawn alternatively from the top, middle and bottom.
- When the seed lot is in bulk, the primary samples shall be drawn from random positions & depths, using a sleeve trier.

Seed Sampling Procedures

- In case of chaffy seeds such as most pasture grasses that are not free flowing, the primary samples may be drawn by hand. Groundnuts, soybeans, fussy cotton, beans and similar crops may also be drawn by hand to avoid damage to the seed.
- All primary samples are then thoroughly mixed into a composite sample from which a submitted sample can be drawn.

Sampling Probes

Sampling Seed Bags

- Probes must reach diagonally across bag
- Short Triers are not advised if not possible to access all parts of the bag

Sampling Procedure:

- Insert probe diagonally at corner
- Keep slots down and closed initially
- Rotate the probe up and twist to fill chambers
- Close chambers and extract the probe
- Seed tumbles out of the mouth of the probe
- Repeat until sufficient sample is obtained

Free Flowing Seed

Prairie Grass Seed

How Many Traditional Bags to Probe?

AOSA recommendation:

5 bags plus 10% of total number, maximum of 30 bags

Total Bag Qty	7	10	23	50	100	200	300	400
Sample Qty	6	6	7	10	15	25	30	30

ISTA recommendation (containers < 100 Kg):

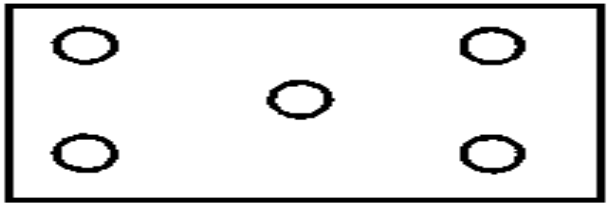
Total Bag Qty	1-4	5-8	9-15	16-30	31-59	60+
Sample Qty	3/bag	2/bag	1/bag	15	20	30

GIPSA recommendation:

36 bags to test for most lots under 10,000

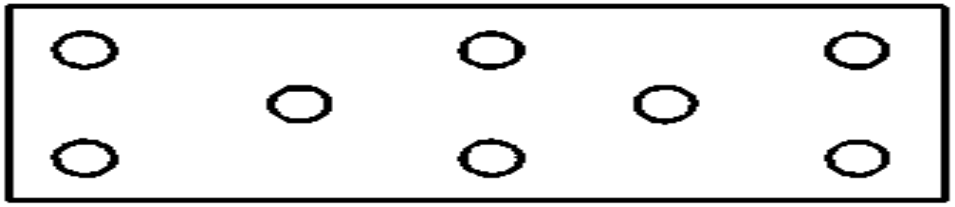
Bulk sampling plan

Lots up to 15 t:
Five sampling points middle and 500mm from sides

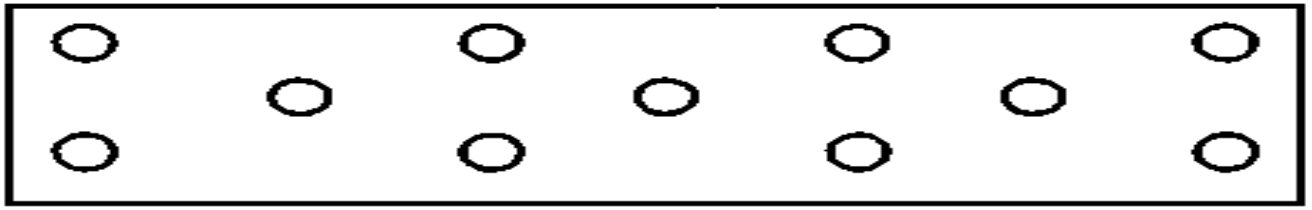


(Wagon truck or hold of ship viewed from above)

Lots from 15 to 30 t:
Eight sampling points



Lots from 30 to 50 t:
Eleven sampling points

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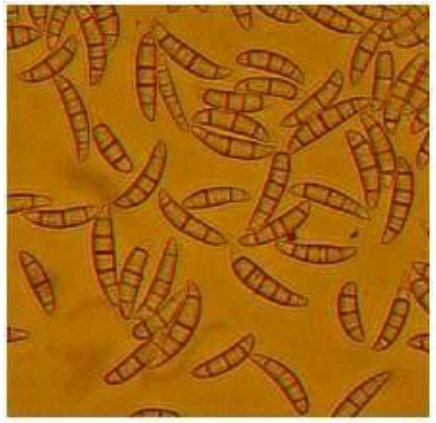
SEED ENTERPRISE MANAGEMENT INSTITUTE (SEMIs)

Seed Quality Assurance, Management and Control Processes

Introduction to Seed Quality and Quality Attributes



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Quality Seed

Seeds of high quality should:

- ✓ be true to its kind or variety,
- ✓ contain a minimum of impurities
- ✓ have high establishment rates in the field (high vigour)

Seed Quality and Seed Quality Attributes

- Deterioration in seed quality may begin at any point in the plant's development stage from fertilization.

They include physical conditions during:

- ✓ growth stages e.g. temperature, nutrition, moisture
- ✓ harvesting,
- ✓ processing,
- ✓ storage
- ✓ planting

Seed Quality Determinants

- Original seed source
- Seed productive method
- Harvesting of seed
- Field contamination
- Seed storage
- Conditioning (processing)

Seed Quality Attributes

- Genetic quality
- Physical purity
- Seed health
- Seed viability
- Seed vigor
- Moisture content
- Testing for traits or unintended presence

Seed Quality and Seed Quality Attributes

- Genetic purity - refers to the trueness to type. The genetic purity has direct effect on ultimate yields
- Physical purity - refers to the physical composition of seed lots.
- Seed germination - refers to the ability of a seed when planted under normal sowing conditions to give rise to a normal seedling.

Seed Quality and Seed Quality Attributes

- Seed viability is the ability of the embryo to germinate and is affected by a number of different conditions
- Seed germination is a process by which a seed embryo develops into a seedling
- Germination involves the reactivation of the metabolic pathways that lead to growth and the emergence of the radicle or seed root and plumule or shoot

- Three fundamental conditions must exist before germination can occur:
  - ✓ The embryo must be alive, called seed viability
  - ✓ Any dormancy requirements that prevent germination must be overcome
  - ✓ The proper environmental conditions must exist for germination

- Seed vigor is a measure of the quality of seed, and involves the viability of the seed, the germination percentage, germination rate and the strength of the seedlings produced

Seed Quality and Seed Quality Attributes

- Planting value - the real worth of a seed lot for raising the crop.
- Pure live seed = Pure seed % X Germination % X 100
- Seed health - refers to the presence or absence of disease organisms/ insect pests on seeds
- Seed moisture - seed moisture is important in the maintenance of seed germination and viability during storage. The seed must be dried to safe moisture content.

Seed Quality and Seed Quality Attributes

High quality seeds are the result of good production practices, which include:

- ✓ proper maintenance of genetic purity
- ✓ good growing conditions
- ✓ proper timing and methods of harvesting
- ✓ appropriate processing during threshing, cleaning and drying
- ✓ appropriate seed storage and seed distribution systems

Factors Affecting Seed Quality

- Seed quality is determined by genetic and physiological characteristics
- Genetic factors that can influence quality include:
  - ✓ genetic make-up,
  - ✓ seed size
  - ✓ bulk density

Seed Quality and Seed Quality Attributes

The physical or environmental characteristics include:

- ✓injury during planting and establishment
- ✓growing conditions during seed development
- ✓nutrition of the mother plant
- ✓physical damage during production or storage by either machine or pest
- ✓moisture and temperature during storage
- ✓age or maturity of seed

Seed Health

How does seed contamination occur?

Seed contamination or infestation

Pathogen itself or parts of it stick or mix with seeds during:

- Harvesting
- Extraction
- Threshing
- Selection
- Packing

Accompanying contamination

Physical mixing of the seed with pathogen's propagation organs

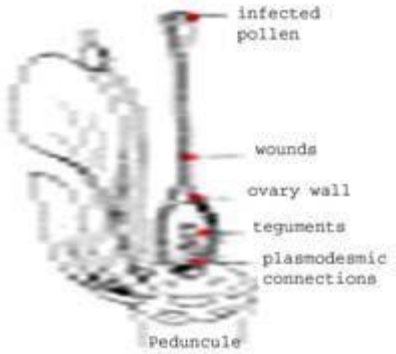
- Spores
- Sclerotium
- Nematode's galls
- Contaminated plant parts or soil particles containing pathogens

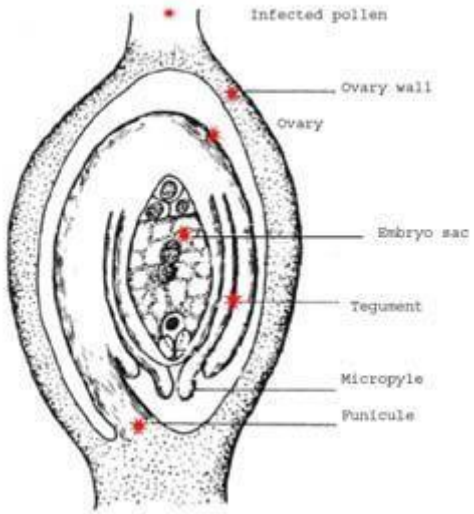
Location of pathogen in seed

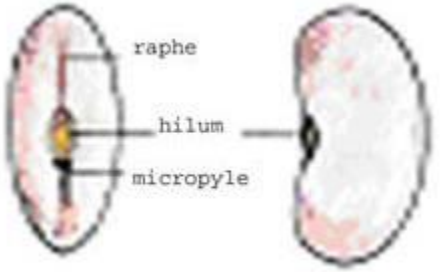
- Infection of the embryo
- Under the seed coat
- In the endosperm or cotyledon
- On the surface of seed

How pathogens infect seed

- Systemic Infection of the Seed
  - Through flowers, fruits or funiculus
  - Through the stigma
  - Through the wall of the ovary or immature seed covers
  - Through wounds & natural openings







- Seed contamination or infestation
  - Pathogens that stick to the surface of the seed

- Accompanying contamination
  - Structures of the pathogens
  - Mix with infected plant parts
  - Soil

The seed borne pathogens may result in:

- ✓ loss in germination
- ✓ discolouration and shrivelling
- ✓ development of plant diseases
- ✓ distribution of pathogen to new areas
- ✓ introduction of new strains or physiologic races of the pathogen along with new germplasm from other countries
- ✓ toxin production in infected seed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DETECTION OF SEEDBORNE PATHOGENS

Methods of detecting seed infection

1. Inspection of dry seeds – detects the presence of fruiting structures of fungi and the effects of fungi on the physical appearance of seeds
2. Blotter methods – this is a simple and inexpensive way to detect seedborne fungi that respond to sporulation
3. Agar plate methods - Detects and identifies seedborne fungi through colony characteristics which they exhibit when grown on nutrient agar
4. Seed washing test - reveals identifiable spores or mycelia adhering to or growing on the seed surface
5. Growing-on test - Detects seedborne fungal, viral, and bacterial pathogens which are readily transmittable.

Requirements

- Untreated and unsorted seed samples purchased from local market (maize, bean, sorghum, millet, cowpea)
- A simple purity box, with a smooth top surface - use white manila paper in absence of purity box Stereomicroscope (magnification at least up to x 50)
- Compound microscope (magnification up to x 400)
- Magnifying lenses
- Balance (up to 3 decimals)
- Disposable plastic Petri dishes
- Glass slides and cover slips
- Sterile distilled water
- Forceps, mounting needles, scalpel blades with handles
- Microscope lens cleaning paper
- Felt pens, paper sticker labels
- Filter papers (9 cm diameter) with high holding capacity
- Trays (30 x 60 cm) for holding Petri dishes
- Beakers
- Alcohol lamps

Inspection of dry seeds

The method provides quick information on insect and mechanical damage to the seeds. The examination of dry seeds is done during the purity test.

1. Select a random sample of 2500 seeds from a well-mixed sample from unsorted seed from local market
2. Place the seed sample on purity board (use white Manila paper in absence of lighted purity board).
3. Using a seed pushing wedge (or a knife, forceps or scalpel blade), separate the seeds intothe following groups based on physical abnormalities (may use magnifying lens):
   - •Shriveled seeds,
   - •Reduction or increase in seed size ,
   - •Discoloration or spots in the seed coat

- Rotten seeds
- Insect damaged seeds

Count and weigh the seeds in each component group and express as percentage.

Using magnifying lens and stereomicroscope observe the fungal structures on rotten, discoursed and shriveled seed (the fungal structures may be mycelia, spores, acervuli, pycnidia, pericthecia, sclerotia on the seed surface or submerged in the seed coat; sclerotia may be loosely mixed with seeds).

Blotter method

1. Select a random sample of 400 seeds from a sample; surface sterilize the seeds in 2.5% sodium hypochlorite, rinse the seeds in sterile distilled water and blot dry on sterile paper towel.
2. Place three 9.0 cm filter papers in each Petri plate and soak with sterile distilled water. Drain away excess water.
3. Aseptically place 10 seeds, evenly spaced, on the surface of the filter paper in each plate. (use 5 seeds for large seeded species – maize/bean/cowpeas; 10 seeds for small seeded species - sorghum/millet).
4. Incubate for 3 days at 25^{0}C in the dark. Care should be taken while handling the dishes in the tray and transferring them to the incubation room so that the plated seeds are not displaced from their original position.
5. Transfer the plates to a freezer and maintain at –20^{0}C for 24 hours. Freezing prevents germination of the seeds.
6. After freezing, incubate for 6 days at 25^{0}C with alternating 12 hr periods of darkness and near U-V (NUV) light. Plates should be approx. 25 cm below the lights and should not be stacked. Light induces sporulation which helps to identify the fungi.
7. After incubation, bring the Petri dishes to the examination area. Examine the seeds under a stereoscopic microscope at x30 for fungal growth and up to x80 magnification for identification of spores and spore-bearing structures.
8. Record the number of infected seeds in each plate.
9. Make a slide preparations of the fruiting structures of the fungi and observe under a compound microscope

Seed treatments may affect the performance of this test. It should only be performed on untreated seed.

Agar Plate Method

In the agar plate method more than one type of fungal colonies are produced. The number can be 3, 4, 5 or even higher depending on the level of infection present in the seeds.

1. Prepare plates of potato dextrose agar (PDA) medium and allow to cool to 45-50^{0}C in water bath (*amend the PDA media with 20ppm streptomycin sulphate to reduce growth of bacteria – the antibiotic solution is added to media after autoclaving and cooling to 45-50^{0}C*).

2. Select a random sample of 400 seeds from a sample; surface sterilize the seeds in 2.5% sodium hypochlorite for 3-5 minutes, rinse the seeds in sterile distilled water and blot dry on sterile paper towel.
3. Dispense the PDA medium into 9 cm plastic Peteri dishes under sterile conditions.
4. Using sterile forceps, plate 5 to 10 seeds on the surface of non-solidified agar medium (5 seed per plate for large seeded varieties – maize/bean/cowpea or 10 seeds per plate for small seeded species – sorghum/millet).
5. Incubate for 3 days at 25^{0}C in the dark. Transfer the plates to a freezer and maintain at - 20^{0}C for 24 hours. Freezing prevents germination of the seeds. After freezing, incubate for 6 days at 25^{0}C with alternating 12 hr periods of darkness and near U-V (NUV) light.
6. Plates should be approx. 25 cm below the lights and should not be stacked. Light induces sporulation which helps to identify the fungi.
7. Examine the fungi growing out from seeds visually and under stereomicroscope to observe colony characters and morphology of sporulating structures. Make slides of the fungal structures and observe under compound microscope. Identify the most frequently
8. occurring fungal colonies present in all Petri dishes, then the second most frequent, then the third most frequent, and so on.
9. Record the counts of the investigated fungi from each dish

Growing-on test (Seedling Symptom Test)

The growing-on test is based on the fact that some of the seed-pathogens are capable of attacking seeds, making them ungerminable resulting in rooting of seeds, and in producing symptoms on young seedlings or even killing the affected seedlings. These effects can be seen if seeds are sown on suitable substrate and seedling grown under environmental conditions which support expression of such effects.

For this test use the germination test materials –

1. Between paper towel
2. In rolled paper towel
3. On sand

For each seed lot tested for germination using each of the above methods, count the number of seeds (express as percentage) showing infection as observed as:

- Colonization of seeds by heavy growth of fungi resulting in loss of germination (seed rotting).
- Symptoms in roots (discoloration, rotting)
- Symptoms in cotyledons, coleoptile, hypocotyls and leaves
- Death of seedlings

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SEED QUALITY ASSURANCE
Fast Green Test

The fast green test is a quick test used to detect pericarp (seed coat) damage in seed corn and sorghum seeds. Fast green used in low concentrations is non toxic to embryos and small seedlings. Briefly exposing seeds to fast green solution and then rinsing off excess solution results in the damage to the surface of seeds becoming apparent. Damage is classified as light (damage to base of seed or small areas away from the embryo), medium (damage extending along either side of the embryo) or severe (damage to seed over the embryo). Stained seeds can be germinated and the normal and abnormal seedlings examined to observe the nature of the damage. Mechanical damage occurs during harvesting, shelling, conditioning and handling of the seed. Weather also can cause seed coat damage such as early frost. The test is especially useful in setting equipment in seed conditioning facilities so as to maximize output while minimizing damage from machinery.

Requirements

1. 1% Fast green solution
2. Maize seeds
3. Tap water
4. Absorbent paper towels
5. Magnifying glass
6. 250 Erlenmeyer flasks

Testing Procedures

•Prepare a 0.1% fast green solution by adding 1.0 gram of fast green to 1000 ml of water (one quart) or mix 0.1 gram of fast green in 100 ml of water (about ¼ pint).

•Place two replicates of 100 randomly selected seeds into 250 ml beakers or similar- sized containers.

•Add enough fast green solution to submerge seeds and swirl occasionally for 15 to 30 second period. Use the 15 second staining period when severe damage is known.

•Pour off fast green solution and rinse seeds under tap water (the solution can be reused).

•Spread each 100 seeds out on absorbent towels and allow air day for ½ hour to one hour.

Note: fast greens will stain most items it comes in contact with such as clothes, hands and counter tops.

Observations

Observe the green-stained seeds for the following damage categories

- **Light**

  - Damage is not apparent and confined to small areas near the point of attachment to the cob, near or at the crown (top) of seed, and away from the embryonic axis.

- Seeds with "bees wings" tip or base of seed will show heavy dye staining. These seeds usually produce normal seedlings.

➢ **Medium**

- Damage is more severe and occurs anywhere on the seed except in the embryonic axis area.
- Seeds with insect damage, **not** associated with the embryonic axis are also included in this classification.

➢

- Seeds in this category usually leach out when planted and more susceptible to attack by soil microorganisms if planted under stressful soil conditions.

Severe

- Damage is directly on or associated with the embryonic axis.
- Seeds with severe pericarp damage near the funiculus are also included. Large areas of missing or extensively damaged pericarp are considered severe damage.
- Seeds with damage to the embryonic axis commonly show abnormal growth of the warm test.
- Seeds with severe pericarp damage usually perform poorly under stressful planting conditions.
- Damage not associated with the embryonic axis. Large areas of missing or extensively damaged pericarp are considered severe damage.

Recording

Record the number of seeds with light, medium and severe damage

GERMINATION/ HEALTH TEST DATA

Sample ID/ Crop	Replicate	Total No. of seeds	Germ. Seeds	Normal seeds	Abnormal seeds	Mouldy seeds	Dead seeds	Seedling with infection
Maize	R1							
	R2							
	R3							
	R4							
	Mean							
Sorghum	R1							
	R2							
	R3							
	R4							
	Mean							
Bean	R1							
	R2							
	R3							
	R4							
	Mean							
Cowpea	R1							
	R2							
	R3							
	R4							
	Mean							
Green gram	R1							
	R2							
	R3							
	R4							
	Mean							
Ground nut	R1							
	R2							
	R3							
	R4							
	Mean							
Lablab	R1							
	R2							
	R3							
	R4							
	Mean							

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GERMINATION TEST

Warm germination tests are used for labeling purposes and give a reasonable idea of field emergence under favorable conditions. A minimum of 400 seeds is required for an AOSA, NSHS, or ISTA official test. Tests can be conducted in rolled brown paper towels (8 reps of 50 seeds), on blue blotter paper, on crepe cellulose paper and on crepe cellulose paper covered with sand. At the end of this period the seedlings are categorized as normal, abnormal, or diseased, and dead or hard seeds. The percentage germination is calculated from the number of normal seedlings from the total number of seeds evaluated.

Requirements for germination test

- Certified and uncertified seeds of maize and bean
- Plastic sandwich boxes with lids
- Absorbent paper towel (kitchen rolls)
- Felt pens
- Paper labels
- Forceps
- Distilled water
- Hand sprayer (to apply water on to paper towels)
- Rubber bands
- Autoclave sterilized river sand

Between wet paper towel

1. Place 3 layers of folded absorbent paper towel in the sandwich boxes; wet the towels thoroughly and tilt the tray up on one end so that excess water runs off the tray.
2. Select a random sample of 100 seeds from the seed lot; count 50 seeds and evenly place on the towel (10 rows of 5 seeds each or 5 rows of 10 seeds each depending of the size of the box).
3. Place 2 layers of wet paper towel above the seeds.
4. Carefully place the lids to prevent drying out and place the boxes at 25^{0}C near diffused light (may place near windows) for 5 to 7 days.
5. After five to seven days, open the boxes and count the number of germinated seed with intact tap roots and shoots. Do not count mouldy seed or diseased seedlings. Testing 400 seeds in this way will give a good indication of the germination percentage.

NB: For each crop variety (maize and bean), prepare at least 15 boxes of uncertified seed and 15 boxes of certified seed (at least 30 boxes for maize and 30 boxes for

In rolled paper towel

1. Wet 3 layers of kitchen paper towel using distilled water.
2. Select a random sample of seed and evenly place 100 seeds on the wetted paper towel (10 rows of 10 seeds each).
3. Place 2 layers of wet paper towel over the seeds, pressing the edges to seal them.
4. Gently roll up the paper towel, making sure the seeds do not drop out. Rolling facilitates handling and helps keep the towel from dying out.
5. One end of the roll is tied with rubber band and the rolls are placed in polyethylene paper bags (rubber band tied side down) – place 6 rolls in each paper bag. The bags are sealed to prevent drying and placed near a window such that the rolls are upright with rubber held side down.
6. After about 5 to 7 days, the rolls are removed, unrolled completely uncovering the germinating seedlings.
7. Ungerminated seeds and germinated seedlings are separated and counted to calculate the germination percentage.

NB: For each crop variety (maize and bean), prepare at least 30 rolls of uncertified seed and 30 rolls of certified seed (at least 60 roll for maize and 60 rolls for bean)

On sand - Duration typically 7-10 days

1. Autoclave-sterilize wet river sand and allow the sand to cool for 2 to 3 days.
2. In each plastic tray evenly spread about 1cm layer of sand and select a random sample of 100 seeds – arrange the 100 seeds in 10 rows of 10 seeds each on the sand.
3. Apply 1 cm layer of sand above the seeds
4. Place the trays in a warm (25^0C) place – near a window and water as required.
5. After 7 to 10 days record germination - count the normal seedlings, abnormal seedling and dead seeds were counted separately and expressed in percentage.

NB: For each crop variety (maize and bean), prepare at least 15 trays of uncertified seed and 15 trays of certified seed (at least 30 trays for maize and 30 trays for bean).

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Hypochlorite Soak Test

The soak test is a quick test to reveal soybean seed coat damage. The test is primarily used during harvest to help reduce mechanical damage due to combining and seed handling. Samples are tested in the field. The test is also useful in determining processes which cause damage within the conditioning plant

Requirements

- Soybean seeds
- 1% sodium hypochlorite solution
- Absorbent paper towels
- 100 ml beakers
- Forceps
- Petri dishes

Procedures

• Prepare a 1% sodium hypochlorite solution.
• Obtain petri dishes which each hold 100 seeds.
• Take a representative sample and four replicates of 100 seeds each.
• Submerge into 1% solution for 10 minutes.
• Remove swollen seeds
• Average the number of swollen seeds per replicate

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THE PURITY TEST

Objectives of the purity test

The object of the purity analysis is to determine the percentage composition by weight of the sample being tested and by inference the composition of the seed lot and to identify the various species of seeds and inert particles constituting the sample.

•To determine the physical composition and quality of a seed lot. The first step is to verify the identification of the species in question.

•Identify the other crop seed, weed seed, and noxious weed seeds.

•To examine the inert material such as soil, insect parts, plant material, and ergot.

•To identify and quantify of any noxious weeds present helps to prevent the spread of noxious weeds.

•To check for and prevent adulteration of seed lots through tolerance testing

Requirements:

- Seeds with contaminating matter (other crop seeds, inert particles, weed seeds, broken seeds)
- Lighted purity board or use white paper for separation (manila paper)
- Hand lenses
- Weighing balances (up to 3 decimal places)
- Knives (scalpel blades) and forceps

Working sample: The submitted sample is mixed and randomly divided into required weight specified in seed rules - 2500 seeds for purity examination.

Component parts: The working sample is divided by hand on a purity board under good light (use white manila paper in absence of lighted purity board), using magnifier light for work with small seeds, tweezers, a seed pushing wedge,and a lot of hand eye coordination.

The component parts are:

1. Pure Seed: Includes all seeds of each kind and/or cultivator under consideration which are present in excess of 5% of the whole.

2. Other Crop Seed: Seed of plants grown as crops (other than the kind or cultivator included in pure) shall be considered other crop seeds, unless recognized as weed seeds by laws, regulations, or by general usage.

3. Weed Seed: Seeds, florets, bulblets, tubers, or sporocarps of plants recognized as weeds by laws, regulation or by general usage shall be considered weed seeds. Further classification of species is determined with the use of the reference, "Uniform Classification of Weed and Crop Seed" which is published by the Association of Official Seed Analysts.

4. Inert Matter: Soil particles, stones, chaff, stems, leaves, flowers, cone scales, pieces of bark, pieces or resin, etc. Pieces of broken and damaged seed units of crops which are half the original size or less. Damaged weed seed with over half the embryo missing.

Each of the four component parts is weighed and a percentage is calculated from the sum of the four component parts.

Purity test reports

The purity test report states the percentage of pure seeds found in the examined prescribed weight. The report will also state what inert matter was found & may include the following:

- Broken seed, soil, scraps
- Other seed: All seed other than the stated species
- Ergot: Ergot is a fungus of the genus Claviceps
- Pests: Any live pests insects etc.

PURITY TEST

Sample ID / Crop	Replicate	Weight of sample	Pure seed	Other crop seed	Inert matter	Weed seeds	Noxious weeds	Germination
Maize	R1							
	R2							
	R3							
	R4							
	Mean							
Sorghum	R1							
	R2							
	R3							
	R4							
	Mean							
Bean	R1							
	R2							
	R3							
	R4							
	Mean							
Cowpea	R1							
	R2							
	R3							
	R4							
	Mean							
Green gram	R1							
	R2							
	R3							
	R4							
	Mean							
Ground nut	R1							
	R2							
	R3							
	R4							
	Mean							
Lablab	R1							
	R2							
	R3							
	R4							
	Mean							

SEMIS SHORT COURSE
SEED QUALITY ASSURANCE
Tetrazolium (TZ) Test

Tetrazolium test is used to detects signs of life or metabolic activity in seeds as an indicator of seed viability (and sometimes vigor). It can also be used to detect frost damage, estimate vigor, or diagnose seed lot problems. Seed are pre-conditioned to take up (imbibe) water slowly. Seed that takes up water too rapidly can rupture cells, giving a false reading. After pre-conditioning, the seed often has to be cut to allow the tetrazolium solution to rapidly move into the seed.

Two reps of 100 seeds (typically) are placed between moist brown paper towels or blotter paper overnight. The next day the seeds are pierced, cut in half, or left whole (depending on species - corn is split in half longitudinally; soybean and bean cotyledons are split) and placed in tetrazolium solution. After a short period of time, the seeds are examined for staining patterns.

The living tissue in the seed (the germ or embryo) turns red within a few hours. The reaction that causes the change in color is related to the respiration rate. Red or pink tissue means that the tissue is healthy and is respiring normally, black that the tissue is respiring rapidly due to either injury or being a meristematic area, and white is dead tissue with no respiration.

•**Viable Seed** - Indicates that a seed contains structures & substances including enzyme systems that give it the capacity to germinate under favorable conditions in the absence of dormancy.

•**Non-viable Seed** - A seed possessing deficiencies and/or other disturbances of such a nature as to prevent development into a normal seedling.

TZ testing is useful in:

- Supplementing germination test results
- Determining dormancy in seed lots
- Diagnosing causes of seed deterioration
- Rating seed lots for vigor
- Quick and reliable information regarding
- Seed viability

TZ Test Evaluation Objective

- Identify those seeds that have the potential to produce normal, viable seedlings.

•Determine which seeds are non-viable and possible causes of deterioration
• Evaluate dormancy after a germination test
• Assess seed soundness, vigor and general health

Preparation of TZ solution

Prepare a 0.1% TZ solution by adding 1.0 gram of tetrazolium to 1000 ml of water or mix 0.1gram of tetrazolium in 100 ml of water.

Phaseolus (Bean)

1. Instruments – beakers (4 x 250ml), razor blades, dissecting needles, scapel, filter paper, forceps, magnifying glasses.
2. Pre-treatment – soak seeds for 18 hours between wet paper at 200C
3. Preparation before staining – leave seed intact
4. Staining – 18 hours, 300C in 1% TZ solution
5. Preparation for evaluation – Remove seed coat to expose embryo
6. Evaluation (maximum area unstained, flaccid and/or necrotic tissue permitted) - ⅔ radical, measured from the radical tip, ½ distal area of cotyledons, ¼ of distal area of plumula
7. Remarks – if the viability of hard seeds is to be determined, the seed coat can be incised at distal end of cotyledons and soaked in water (4 hours).

Zea mays (maize)

1. Instruments – beakers (4x250 ml), razorblades, dissecting needles, scalpel, filter paper, forceps.
2. Pre-treatment – soak seeds for 18 hours between wet paper towel at 200C.
3. Preparation before staining – cut longitudinally through embryo and ¾ of endosperm.
4. Staining – 2 hours at 300C in 1% TZ solution.
5. Preparation for evaluation – spread into two halves, observe cut surface.
6. Evaluation (maximum area of unstained, flaccid and/or necrotic tissue permitted)
7. Remarks – Unstained tissue at centre of scutellum is indicative of head damage.

Reporting of TZ results - Based upon the colour and location of dead or injured tissue, a potential germination percentage can be determined.

Classification of Seeds as Non-Viable

• Evidence of necrosis or decay

•Half or more of the total cotyledon tissue in dicots non-functional
• Critical connective tissues damaged or decayed
• Flaccid tissues
• Pathogen invasion
• Mechanical breaks or bruises, especially in locations that would impair growth and development

TZ as a Vigor Test

• Seeds placed in categories based on:
• Intensity of TZ staining
• Location of dead and/ or deteriorated tissue
• Amount of dead or dying tissue
• Development of the embryo
• Categories estimating vigor as the seed is being evaluated for germination:
➢High vigor
➢Medium vigor
➢Low vigor
➢Non-germinable (dead seed)

The living tissue in the seed (the germ or embryo) turns red within a few hours. The reaction that causes the change in colour is related to the respiration rate. Red or pink tissue means that the tissue is healthy and is respiring normally, black that the tissue is respiring rapidly due to either injury or being a meristematic area, and white is dead tissue with no respiration