National Workshop on Seed Testing

(September 04-06, 2023)

Workshop Manual

Organized by:

Government of India
Ministry of Agriculture & Farmers Welfare
Department of Agriculture & Farmers Welfare
National Seed Research & Training Centre
GT Road, Collectry Farm, Varanasi

भारत सरकार
राष्ट्रीय बीज अनुसंधान एवं प्रशिक्षण केन्द्र
कृषि एवं किसान कल्याण मंत्रालय
कृषि एवं किसान कल्याण विभाग
जी. टी. रोड, कलेक्ट्री फार्म
पोस्ट आफिस इन्डस्ट्रीयल इस्टेट
वाराणसी – २२१ १०६ (उ.प्र.)

GOVERNMENT OF INDIA
NATIONAL SEED RESEARCH & TRAINING CENTRE
Ministry of Agriculture & Farmers Welfare
Department of Agriculture & Farmers Welfare
G.T. Road, Collectry Farm,
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Varanasi- 221 106 (U. P)

FORE WORD

Seed is the most vital input in sustainable agricultural production. It is well known fact that increases in crop yield could be achieved by use of quality seeds. Ensuring the quality of seeds is very important and therefore the significance of seed testing becomes imperative for success of any seed production programme.

It gives me immense pleasure to inform that National Seed Research and Training Centre, Varanasi is organizing the **National Workshop on "Seed Testing"** during **September 04-06, 2023** at NSRTC, Varanasi. This National Workshop is of utmost important to augment the income of farming community and agricultural production through the availability of quality seeds across the country.

The main objective of this National Workshop is to update the knowledge, skills and to provide hands on experience to the personnel working in the field of seed testing and seed quality control of seeds. This National Workshop will be very useful in obtaining uniformity in seed testing, seed quality regulation and exchange of information in the area of seed testing at national level.

This programme shall provide technical inputs on various aspects of seed testing by experts who are having enriched experience in their field. The National Workshop manual is designed to provide updated information on seed testing on various seed related aspects. It will also help in maintaining the uniformity in the seed testing in the country.

I hope that this workshop module will be beneficial to all participants.

(Manoj Kumar, IAS)
Director

Date: 06.09.2023
Place: Varanasi

National Workshop
On
SEED TESTING
(September 04-06, 2023)

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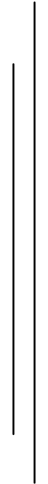

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National Workshop on "Seed Testing"

(September 04– 06, 2023)

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NSRTC at a glance...

National Seed Research and Training Centre (NSRTC), Varanasi established under Govt. of India, Ministry of Agriculture& Farmers Welfare, Department of Agriculture and Farmers Welfare, during October 2005.

The prime objective of establishment NSRTC is to have a separate National Seed Quality Control Laboratory, which is serving as **Central Seed Testing Laboratory (CSTL)** as well as to act as **Referral laboratory** for hon'ble court of the entire country.

Further, this **CSTL** has to coordinate and monitor the functioning of all the **notified State Seed Testing Laboratories** presently available in our country in order to obtain Uniformity in Seed quality Regulation at National level.

More importantly for facilitating International seed Movement, our CSTL the member laboratory of International Seed Testing Association (ISTA), ZURICH, Switzerland and expected to become accreditated Laboratory very soon and thereafter will be eligible for issuing International seed movement certificates on behalf of Government of India.

NSRTC is the National Centre for Training Human resources for the officials who are all involved in the **Seed Quality Control, Seed Law Enforcement and stake holders of Seed Industry.**

In order to fulfill the mandate, NSRTC organize National trainings, workshops, National seed congress for the benefit of personnel involved in seed development and quality control programme and stakeholders of seed industry for updating their knowledge and skills.

The NSRTC is situated under greater periphery of the Holy city Varanasi, which is located 7 KM away from heart of city towards south – west on Varanasi - Allahabad GT road, Collectry farm, surrounded by Banaras Hindu University (6 km), Indian Institute of Vegetable Research (20kms) and well linked by Air, Train and Road.

PRIME OBJECTIVES:

- To have a separate National Seed Quality Control Laboratory, which is serving as **Central Seed Testing Laboratory (CSTL).**
- To act as **Referral laboratory** for hon'ble court for the entire country w.e.f 1.4.2007 onwards.
- Member laboratory of **International Seed Testing Association (ISTA)**, Switzerland,
- Centre for testing all transgenic crop seeds etc., in future
- **To organize National and International seed related conferences, symposium and trainings** for the benefit of personnel who are involved in seed development and quality control programme and stakeholders of seed industry.
- Centre for training human resource on all seed related aspects.

VISION:

Our vision is to

- Contribute integrated approach towards quality seed availability.

- Have separate National Seed Quality Control Laboratory as CSTL.
- Maintain uniformity in seed testing and seed quality control at National level.
- Make Seed Industry in India globally competitive.

MISSION:

Our mission is to lead and engage in downstream programmes on Seed Science and Quality Control to disseminate the values of seed production and availability of quality seed to the need of National and International seed community.

STRATEGY:

NSRTC pursues its Mission and Goals through

- Integrated approach and system –based programs on seed quality control and act as Referral Lab for the hon'ble Court.
- Strengthening Seed Technological Research in seed production disciplines of major crops.
- Total seed quality management through systemic seed certification and law enforcement process.
- Interaction with stake holders of seed industry, officials of seed certification and law enforcement, seed producers and other seed organizations that share's NSRTC mission.
- Continued efforts in improving / updating knowledge and skill of human resources involved in seed certification and quality control as a training human resource on all seed related aspects
- In order to meet out these vision and missions strategy the NSRTC is housed in a modern building with all latest infrastructural facilities, equipments and machineries, excellent conference/ seminar hall, workshop /class rooms, exclusive ISTA member laboratories, museum, well stocked library.

Staff strength:

The Ministry of Finance sanctioned of 23 posts for National Seed Research and Training Centre, Varanasi for making the centre functional so as to meet out the mandate.

NSRTC is especially designed for continuous dissemination of knowledge of seed and thereby improve skill, competency and scientific soundness of individuals engaged in seed development programme. NSRTC regularly organizes training on various aspects of seed for the officials working in Seed Certification Agencies (25 in number), Seed Testing Laboratory (147 in number), Seed Law Enforcement Agencies, Agricultural Universities and other institutes dealing with seeds. The NSRTC, Central Seed Testing Laboratory acts as a referral lab under clause 4(1) of the Seeds Act, 1966. CSTL, NSRTC is testing more than 20,000 samples per year and performs at par with ISTA (International Seed Testing Association) with regard to seed testing net work in the country.

National Seed Testing Laboratory as Central Seed Testing Laboratory

The testing of seed material will be flowing from different State Seed Corporations as well as Seed Producing Organizations for physical purity, seed health and at later stage genetic

purity that is mostly required in referral cases. At present the mandate of Central Seed Testing Laboratory (CSTL) is to receive 5% samples from seed producing organizations all over the country. In addition, CSTL act as a Nodal centre for coordinating the activities of Seed Quality Control programmes on behalf of Government of India in accordance with the Act and Rules with the State Notified Seed Testing Laboratories.

Grow Out Test

NSRTC have been allotted 10 hectares of land out of which the office premises have been constructed in about 2.5 hectares of land and remaining land have been kept reserve for organizing Grow Out Test for which Green House/Poly House and other necessary facilities have been created.

NSRTC is geared to go Global

NSRTC is a globally competitive Institute in Seed Science and Quality control, marching ahead with:

- To promote the availability of quality seed to meet the challenges of Science based Agriculture.
- Making of promising Technologies reach the seed entrepreneurs and other stakeholders through innovative Trainings, Conferences, Workshops and Symposia.
- Establishing uniformity in Seed production & Quality Control programmes at National level.
- Innovative curriculum planning and implementation to make Seed Science & Research more vibrant and responsible to match the vision and needs of present and future.

Manoj Kumar, IAS
Director, NSRTC

Principles and Procedures of Seed Sampling Techniques

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The reliability of the inference made about the quality of the seed lot depends primarily on two components: the accuracy with which the sample represents the lot and the accuracy and precision of the laboratory test. It is observed in many cases that the variations in test results are due to the variation in the sampling. Hence, seed sampling is one of the basic components responsible for the accurate Seed Testing results. Therefore utmost care is required for drawing the sample No matter how accurately the laboratory tests are done, the results can only show the quality of the sample submitted for analysis; consequently the sample should accurately represent the composition of the seed lot.

Objective

The object of sampling is to obtain a representative sample of a size suitable for test. When the sample originates from a seed lot, it is expected that the test results reflect the average quality of the seed lot

Sampling

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

Seed Lot

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

Primary Sample

Small portion taken from one point in the lot as shown in the fig below.

Composite Sample

Is formed by combining and mixing all primary samples taken from the lot.

Submitted Sample

Sample submitted to seed testing laboratory. The size of the submitted sample is specified in the Seed Testing Rules.

Working Sample

Is a sub sample taken from Submitted Sample in the laboratory, on which one of the quality tests is made.

General Principles of Sampling

Under seed law enforcement programme only trained and experienced officials are authorized to undertake sampling and he has to:

Give notice to such intention to the person from whom he intends to take sample.
Take three representative samples in the prescribed manner and mark & seal.

- One sample to be delivered to the person from whom it has been taken
- Second to be sent for analysis to the Seed Analyst of the area.
- Third to be retained for any legal proceedings.
- At least two persons should be present and obtain the signature of both the witnesses on form VIII of the Seed Rules.
- Sampler must verify the information provided on the label as per the requirements of the Seed Act:

Following information should be checked on label

- Kind
- Variety
- Lot Number
- Date of Test
- Seller's name & address

In case of certified lots sampler should check Information on seed certification tag:

- Name & Address of certification agency
- Kind & Variety
- Lot No.
- Name a& Address of certified seed producer
- Date of issue of the certificate & its validity
- Class & Designation of seed
- Period during which the seed shall be used for sowing

The seed lot should be so arranged that each individual Or part of the lot is conveniently accessible.

Upon the request of the sampler, the owner shall Provide full information regarding bulking and mixing of the lot. When there is definite evidence of heterogeneity sampling shall be refused. If the nature of the presentation of the seed lot or container makes it impossible to adequately apply these procedures, then the sampling Shall not be undertaken, and alternative presentation of the seed lot should be sought.
The size of the seed lot should not exceed to the maximum size as prescribed in the rules subject to 5% of tolerance.

Minimum Sampling Intensity for Seed Lots when seed in bulk

Upto 500	***At least 5 primary samples***
501 –3,000	One primary sample for each 300 kg but not less than 5
3,001 – 20,000	One primary sample for each 500 kg but not less than 10
20,001 or more	One primary sample for each 700 kg but not less than 40

Minimum Sampling Intensity For seed lots in containers

Containers	**No. of Primary Samples**
1 – 4	3 samples from each container.
5 – 8	2 samples from each container

9 – 15	1 samples from each container
16 – 30	15 samples in total
31 – 59	20 samples in total
60 or more	30 samples in total

For seed lots in containers smaller than 15 kg capacity, containers shall be combined to sampling units not exceeding 100kg and sampling units shall be regarding as containers in the sampling scheme above.

Equipments for Sampling

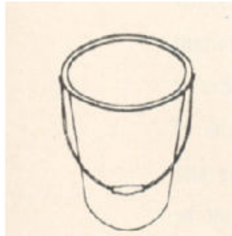
Sleeve or Stick type trier (Bag trier): The tube and sleeve have open slots in their walls So that when the tube is turned until the slots in tube and sleeve are in line. Seed can flow into the cavity of the tube, and when the tube is given half turn the opening are close.

Bin trier: It is larger than sleeve trier constructed on the same principle. It is used for sampling from heaps and godowns.

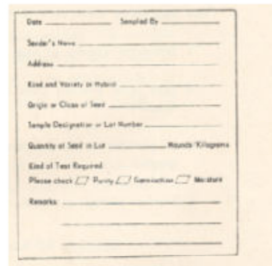
It consist of hollow tube inside, closely fitting outer sleeve has solid pointed end.

Nobbe trier: Pointed tube long enough to reach the center of the bag with an oval hole near the pointed end.

Other items needed while sampling

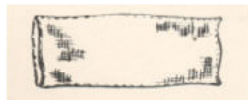


Bucket or pan for collecting individual trier samples



Date ________ Sampled By ________
Sender's Name ________
Address ________
Kind and Variety or Hybrid ________
Origin or Class of Seed ________
Sample Designation or Lot Number ________
Quantity of Seed in Lot ________ Pounds/Kilograms
Kind of Test Required
Please check ☐ Purity ☐ Germination ☐ Moisture
Remarks ________

Label on which detailed information about seed lot to be put inside bag



Cloth bag for holding samples

Address label to be attached To the bag

Alkathene bag to hold 150 gms of seed

Method of obtaining primary samples

Steps to follow

1. Check lot and count bags

2. Insert the Trier in Right Direction

Methods for obtaining working samples

A. Sampling in Seed Testing Laboratory

Seed samples received in the Seed Testing Laboratory e in the Seed Testing Laboratory (submitted sample) are required to be reduced to obtain working samples for carrying out various test.

Soil Divider

It is simpler divider, built on the same principle as the conical divider, is the soil divider. The channels are here arranged in a straight row instead of a circle as in the conical divider. The soil divider consists of a hopper with attached channels or ducts, a frame to hold the hopper, two receiving pans and a pouring pan.

The following dimensions have been found suitable. Ducts or channels, 12.7 mm wide, lead from the hopper to the collecting pans. There are 18 of these channels, alternate ones leading to opposite sides. The maximum dimensions are: 35.6 cm long; 25.4 cm wide; and 27.9 cm high.

In using the divider the seed is scattered fairly evenly in a pouring pan the length of the hopper and poured in at approximately equal rates along the entire length of the hopper. This divider is suitable for large-seeded and chaffy species, but suitable types for small seeded species can also be made.

Conical divider

Suitable dimensions:

Large divider, designed for large seeds and grains, there are 19 channels and 19 spaces, each 25.4 mm wide.

Small divider designed for small free-flowing seeds there are 22 channels and 22 spaces, each 7.9 mm wide.

The overall dimensions of the dividers are

large divider, 81.28 cm high and 36.83 cm in diameter;

small divider, 40.64 cm high and 15.24 cm in diameter.

Conical divider or Bouerner type seed divider

Centrifugal divider

The centrifugal divider (Gamete type) makes use of centrifugal force to mix and scatter the seeds over the dividing surface. In this divider the seed flows downward through a hopper onto a shallow rubber cup or spinner. Upon rotation of the spinner by an electric motor the seeds are thrown out by centrifugal force and fall downward. The circle or area where the seeds fall is equally divided into two parts by a stationary baffle so that approximately half the seeds fall in one spout and half in the other spout.

Centrifugal Divider or Gamet Type Divider

The centrifugal divider tends to give variable results when not carefully operated.

To avoid variability in the results the divider is operated

i. leveled by means of the adjustable feet.

ii. divider & four containers are checked for cleanliness.

iii. A container is placed under each spout.

iv. The whole sample is fed into the hopper; when filling the hopper, seed must always be poured centrally.

v. The spinner is operated and the seed passes into the containers.

vi. Full containers are replaced by empty containers. The contents of the two full containers are fed into the hopper together, the seed being allowed to blend as it flows in. The spinner is operated.

vii. The procedure described in (vi) above is repeated at least once more.

Spoon method

Useful for samples of a single small-seeded species and for sample reduction for moisture determination or seed health testing.

A tray, a spatula and a spoon with a straight edge are required. After preliminary mixing, pour the seed evenly over the tray; do not shake the tray thereafter.

With the spoon in one hand, the spatula in the other, and using both, remove small portions of seed from not less than five random places. Sufficient portions of seed are taken to constitute a sub-sample of approximately, but not less than, the required size.

Hand halving method

In the International Rules this method is the most satisfactory method for chaffy and genera of tree and shrub seed:

Technique:

1. The seed is poured evenly onto a smooth clean surface.
2. Thoroughly mix the seed into a mound with a flat-edged spatula

3. The mound is divided into half and each half is halved again, giving four portions. Each of the four portions is halved again giving eight portions which should be arranged in two rows of four.

4. Combine and retain alternate portions: e.g. combine the first and third portions in the first row with the second and fourth in the second row. Remove the remaining four portions.

5. Steps two, three and four are repeated using the retained portions from step four until the weight of sample required is obtained.

Random Cups Method

A: Schematic drawing of the tray, the cups and how to distribute the seed over the tray
B: A tray with cups and distributing a sample over the tray.
C: The tray with the total sample distributed over the tray.
D: The cups removed from the tray and emptied into a glass vessel.
E: Cups of different size in one set.

Lot & sample weights of important cereal crops

Crop	**Max. Wt of Seed lot (kg)**	**Minimum Wt. Of sample (g)**	
		Submitted	Working
Paddy	20,000	400	60
Wheat	20,000	1,000	120
Barley	20,000	1,000	120
Pearl millet	10,000	150	15
Sorghum	10,000	900	90
Maize	40,000	1,000	900
Oats	20,000	1,000	120

Lot & sample weights of important pulse crops

Crop	**Max. Wt of Seed lot (kg)**	**Minimum Wt. Of sample (g)**	
		Submitted	**Working**
Black Gram	20,000	1,000	150
Horse Gram	20,000	500	500
Lentil	10,000	600	60
Pea	20,000	1,000	900
Pigeon pea	20,000	1,000	300
Green gram	20,000	1,000	120

Lot & sample weights of important Oil seed & fiber crops

Crop	**Max. Wt of Seed lot (kg)**	**Minimum Wt. Of sample (g)**	
		Submitted	**Working**
Groundnut (pods)	20,000	1,000	1,000
Groundnut kernels	20,000	1,000	600

Mustard	10,000	160	16
Soybean	20,000	1,000	500
Sunflower	20,000	1,000	250
Sunflower Hybrid	20,000	250	125
Safflower	10,000	160	16
Cotton	20,000	1,000	350
Jute	10,000	150	15

Lot & sample weights of important Vegetable crops

Crop	**Max. Wt of Seed lot (kg)**	**Minimum Wt. Of sample (gms)**	
		Submitted	**Working**
French bean	20,000	1,000	700
Bottle gourd	20,000	700	70
Cucumber	10,000	150	70
Tomato	10,000	70	07
Tomato (hybrid)	10,000	07	07
Onion	10,000	80	80
Lettuce	10,000	30	03
Carrot	10,000	30	03
Cabbage	10,000	100	10
Okra	20,000	100	140
Brinjal	10,000	150	15
Cauliflower	10,000	100	10
Reddish	10,000	300	30
Spinach	10,000	500	50
Methi	10,000	40	4

*****

Testing of Vigorsity of Seed Lot

Sangita Yadav, Principal Scientist
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The importance of high seed vigour and viability, to ensure a satisfactory stand establishment and subsequent crop growth is well established. Though the standard germination test is the most useful and commonly performed test to assess the ability of seeds to germinate, the fact that such tests are conducted under most favourable (optimum) conditions ensures that any seed that is capable of germination does so (Powell and Matthews, 1992). Thus, the ability of a seed lot to produce normal seedlings under sub-optimal field conditions do not always match the germinability observed in a laboratory test. There are several reports of seed lots having similar laboratory germination, which differed significantly in their ability to emerge under field conditions. Thus, the concept of seed vigour emerged with the need to assess the field potential of the "Planting value" of a seed lot in a more realistic manner. The initial concepts of vigor focused on the advantages over the germination and Tetrazolium tests with respect to the identification of seed lots able to achieve rapid and uniform emergence and stand establishment under less favorable environmental conditions. Some years later, it was assumed that seed vigor might be considered as a potential for seedling establishment in the field and the same idea was supported by various seed technologists.

International Seed Testing Association agreed to adopt a very broad definition that is "Seed vigour is the sum total of those properties of the seed which determine the level of activity and performance of the seed or seed lot during germination and seedling emergence" (Perry 1978). Later in 1980 Association of Official Seed Analysts adopted the seed vigour definition as "those seed properties which determine the potential for rapid, uniform emergence, and development of normal seedlings under a wide range of field conditions" (AOSA, 1983). Later ISTA has added the wide range of environments to its vigour definition, current definition is "Seed vigour is the sum of those properties that determine the activity and performance of seed lots of acceptable germination in a wide range of environments"**(**ISTA, 2022**)**.

These current accepted concepts do not include the possible persistence of the effects of seed vigor throughout the plant development cycle persisting until yield. It may well be that the emergence of vigorous seedlings can result in high crop productivity because of rapid and uniform stand establishment either under unfavorable situations or after storage.

Seed Vigor Testing

Over the years, many seed vigour tests have been developed and evaluated. A simple classification based on is either a direct or an indirect test is given in Table 1. Sometimes a combination of tests is used on a seed lot to read its planting potential more accurately.

Table 1: Classification of seed vigour tests

Direct Test	**Indirect Test**	
Seedling performance	**Stress based**	**Biochemical based**

based		
First Count	Cool germination test	Conductivity test
Speed of germination	Coid germination test	Tetrazolium test
Seedling growth rate and dry weight	Hiltner test (Brick gravel test)	GADA test
	Paper piercing test	Respiratory Quotient test
	Accelerated ageing (AA) test	
	Controlled deterioration test	

ISTA accepted vigour tests

The International Seed Testing Association has finalized five tests for specific crop species (Table 2) to know their vigour after validation of the tests. The vigour tests provide additional information to the standard germination test to assist in the differentiation of seed lots of acceptable germination.

Table 2: ISTA validated vigour tests

Vigour test	**Species**
Conductivity test	*Cicer arietinum*, *Glycine max*, *Phaseolus vulgaris*, *Pisum sativum* (garden peas only, excluding petit-pois varieties), *Raphanus sativus*
Accelerated ageing test	*Glycine max*
Controlled deterioration test	*Brassica* spp.
Radicle emergence test	*Zea mays, Brassica napus*, *Raphanus sativus, Triticum aestivum*
Tetrazolium vigour test	*Glycine max*

A seed vigour test is either a direct or an indirect analytical procedure to evaluate the vigour of a seed lot under standardized conditions.

I. DIRECT TEST:

Direct tests reproduce environmental stresses or other conditions in the laboratory, and the percentage and/or rate of seedling emergence are recorded. This include seedling performance based test as follows:

Seedling performance based

These tests are based on the assessment of germination and seedling growth and are generally an extension of germination tests. Vigour is measured by recording some additional parameter over the standard germination data. The advantage of such tests is that seed testing laboratories do not require additional equipment and that little additional training of staff is required. The disadvantage is that variables such as humidity and temperature, which exert a significant influence on seedling growth, are difficult to standardize.

A. First Count: It is to be used to compare different seed lots over several months. the higher the percentage of normal seedling at first count, the better the seed quality. The early development of normal seedlings indicates the vigour of the seeds under test.

B. Speed of Germination: Speed of germination is considered one of the oldest seed vigor manifestations. Rapid germination is an important component of the seed vigor concept since it usually corresponds to more rapid seedling emergence in the field. The importance of this parameter for differentiating seed lots according to physiological potential was recognized around.

A more detailed test under the Standard Germination Test, where the observation on the number of seeds germinated has to be recorded at approximately the same time daily. Normal seedlings are removed from the test when they reach a predetermined structure that is capable of producing a normal seedling. An index is computed for each seed lot by dividing the number of normal seedlings removed each day after planting on which they were removed. For e.g. the quality indexes of Lot A and Lot B is obtained in the following manner:

Lot A = No. of Seedling removed Day after planting = 0/1 + 0/2 + 0/3 + 8/4 + 10/5 + 24/6 + 28/7 + 24/8 = 0+0+0+2+2+4+4+3 = 15

Lot B = No. of Seedling removed Day after planting = 0/1 + 0/2 + 12/3 + 24/4 + 45/5 + 7/6 = 0+0+4+6+9+1 = 20

Lot B with a higher index would be considered the better quality lot.

C. Seedling Growth Rate and Seedling Dry Weight: Measurement of seedling growth rate greenhouse or field or laboratory conditions on a specified number of days from planting indicates its vigour. The blotters along with planted seeds are placed in a seed germinator cabinet at 200C in dark, for a fixed number of days. The length of root and shoot of the normal seedlings are recorded, and the seed lot producing the most growth is considered the best quality. The seedlings are dried at 110^{0}C for 17±1 hrs to record their dry weight. Better quality is considered to produce higher seedling dry weight.

D. Vigour indices: These are the most used vigour tests. The seedling length and dry weight in combination with germination gives better vigour understanding.

Vigour index-I = germination (%) X Seedling length (cm)

Vigour index-II = germination (%) X seedling dry weight (mg)

There are no units to vigour index-I and II. These are abstract values and are used as comparative indicators of vigour of seed lots.

E. Radicle emergence (RE) test: This is the new vigour test that was approved by the ISTA in four crop species after validation. The slow rate of germination is an early physiological expression of seed ageing, the major cause of reduced vigour. High radicle emergence, early in germination is indicative of high vigour.

Two hundred seeds are kept for germination using paper medium at recommended temperature for the prescribed period based on the species under test (Table 3). After the

emergence of radicle (as per criteria mentioned in table 3) the number of seeds were counted, and percentage was calculated.

Table 3: Conditions for radicle emergence test

Species	Medium*	Replications	Temperature	Criteria for RE	Timing of RE count
B. napus	PP	2 X 100 seed	20 ± 1^0C	Appearance of radicle	30h±15min
R. sativus	TP	4 X 50 seed	20 ± 1^0C	2 mm radicle	48h±15min
Z. mays	BP	8 X 25 seed	20 ± 1^0C	2 mm radicle	66h±1 min
T.aestivum	BP	4 X 50 seed	15 ± 1^0C	2 mm radicle	48h±15min

* PP= Pleated paper; TP= Top of the paper; BP= Between paper

II. INDIRECT TESTS :

Indirect tests measure other characteristics of the seed that have proved to be associated with some aspect of seedling performance. It includes stress based and biochemical based test

A. Stress based tests

The following tests are stress tests which impose stress conditions on the seeds and based on the performance of the seeds under stress conditions the quality of seed lot in terms of vigour is measured. Among several stress tests ISTA has validated only two tests viz., accelerated ageing test and controlled deterioration test.

1. **Cold germination test:** This test was developed in USA to evaluate seed vigour in Maize. In USA when maize sown in the late spring season when the soil is humid and cold and hampers the growth of weak seedlings. It is the most widely used vigour test for maize and other crops (soybean, sorghum) and is also widely accepted by the seed industry in other parts of the world. The cold test is one of the oldest vigour tests.

The cold test determines the ability of seeds to germinate and produce normal seedlings under two stress conditions viz. a sub-optimal temperature (Cold moist conditions) and pathogen (soil borne pathogens). At low temperatures, low vigour seeds tend to leak out more sugars, amino acids, etc. and encourage growth of pathogen. The ability to perform well in cold wet soils is influenced by genotype, mechanical damage, seed treatment and physiological condition of the seed, the cold test determines the combined influence of these, and possibly other, factors.

In this test the seeds are incubated at 10^0C for 7 days in a moist medium containing soil originating from a maize field. After transfer to a temperature of 25^0C, a count of normal seedlings is made 4 to 7 days later.

2. **Cool germination test:** The test is limited to measuring the effect of cool temperature on the germination of cotton seed and the growth rate of cotton seedlings. Germination test

is conducted at constant temperature of 18^0C and sufficient humidity. Only one count is recorded, on sixth day for acid delinted cotton seed and on the seventh day for machine delinted cotton seed.

3. **The Hiltner Test or Brick gravel test:** The test was originally developed by Hiltner and Ihssen (1911) for seed-borne infection by *Fusarium* spp. after it was observed that coleoptiles from infected, germinated seeds were short and not able to penetrate a 3 cm thick layer of brick grit without physical damage. This method is suitable to study the vigour levels of cereal seeds. Seeds infected by pathogenic fungi, injured seeds, or those low in vigour are often weak and unable to withstand adverse conditions during germination and field emergence. The brick grit layer used in the Hiltner test imposes a physical stress on the seeds. Seedlings which emerge normally through brick grit are considered to have been able to withstand the physical stresses involved, and the test thus provides a method to screen seed lots for vigour.

4. **Paper piercing test:** This test is similar to Hiltner brick gravel test. High vigour seeds produce strong seedlings which can pierce paper. This method utilizes regular testing sand plus a special type of paper disc which seedlings must penetrate to be considered strong. The paper must have the following characteristics: - Basic weight : 90g/m2 - Thickness : 0.4mm - Dry Bursting Strength : 0.3 kg/cm2 - Wet Bursting Strength : 150 mm - Breaking Strength : 1000-5000 mm - Filtering Speed : 500 ml/min - Ash content : 0.1 % The test is used for cereal crops requires placing seed on top of approximately 1.2 cm of moist sand, covering seeds with a special dry filter paper, and covering the paper with about 3 cm of moist sand. Test is carried out at 20^0C for eight days.

5. **Accelerated Ageing test:** The accelerated ageing test was developed in Mississippi State University, USA by Delouche and Baskin (1973). The ageing process is accelerated by subjecting the seeds to elevated temperature (40-45^0C) and high relative humidity (greater than 90%) in a chamber before standard germination. Under these conditions seed deterioration is accelerated causing least damage to high vigour seeds. It also gives indication of performance of seed lot under storage. This is ISTA recommended vigour test for *Glycine max* (Soybean) (ISTA, 2022)
Two replications of 42 ± 0.5 g seed (total approx. 200 seeds for two replications) were placed on the dry screen in the Accelerated ageing box filled with distilled/ deionised water. The Ageing box is placed in the incubator at 41 ± 0.3°C for 72 hours. The seeds should not touch the water surface. After the ageing, the seeds were germinated immediately using the standard germination procedure at recommended temperature and RH for required duration. The percent of normal seedlings was measured at the end of the test. Higher the germination percentage, greater is the vigour of seeds.A saturated salt AA (SSAA) test is used for small-seeded species and sweet corn as it slows moisture uptake typically reducing fungal problems.

6. **Controlled deterioration test:** This vigour test was developed by Matthews (1980). This is similar to accelerated ageing test, except that seeds are pre- conditioned to a specified moisture content (say, 20 %). The storage potential and field emergence of seed

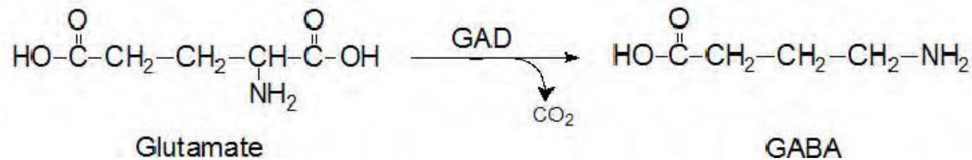
can be determined by subjecting them to high temperature after increasing their moisture content to 20%. High vigour seeds retain high germination after deterioration. This is ISTA recommended vigour test for Brassica sp (ISTA, 2022).

Raise the moisture content of seeds to 20% and then keep at 45°C for 24 h. The seed moisture content was raised by placing 100 seeds of known moisture content on moist filter papers until the required moisture is reached or by adding required quantity of water to the seeds kept in vials. The seeds with high moisture content (approx. 20%) were sealed in aluminium pouches/ 700-gauge polythene bags and kept at 7±2°C for overnight to equilibrate the moisture throughout the seed. After the moisture equilibration, four replicates of seed packets were placed in a water bath at 45°C for 24 h ± 15 min after which the germination test is conducted. In this test both the total germinated seed percentage and percentage of normal seedlings are reported. This method is incredibly useful to correlate the field emergence and storage potential of vegetable seeds (Matthews, 1980). Higher the percent of seeds germinated greater is the vigour as well as storage potential.

B. Biochemical Tests

Radicle emergence and subsequent seedling growth are the end result of metabolic reactions. The biochemical testsdetermines the metabolic activity that occurs during seed deterioration and should provide a measure of seed vigour. Biochemical tests are more rapid than other vigour tests but require specialized equipment and training These are indirect vigour estimation tests. Of the different biochemical tests the conductivity test in *Glycine max, Phaseolus vulgaris, Cicer arietinum, Pisum sativum* (garden peas only, excluding petit-pois varieties), *Raphanus sativus* and tetrazolium vigour test in *Glycine max* is validated by ISTA.

1. **GADA Test:** The Glutamic acid decarboxylase activity (GADA) test was developed by Grabe (1964).It measures the activity of glutamic acid decarboxylase (GAD) enzyme which is a key enzyme during germination helps in estimation of seed vigour . Seed proteins are hydrolyzed into amino acids during germination Glutamic acid comprises a high percentage of the total amino acids in seeds. The level of enzyme activity is determined by the amount of carbon dioxide (CO_2) given off and is positively related to seed quality; i.e., the more the CO_2 evolved, the better the seed quality.



2. **Tetrazolium (TZ) Test:** Tetrazolium test was basically considered a quick viability test. Tetrazolium salt (2,3,5-triphenyl tetrazolium chloride) is colorless . It reacts with hydrogen released during respiration by dehydrogenase enzyme to form water-insoluble formazan (red). Based on colour intensity of stained embryo or seed, it is used for the vigour estimation of seeds. ISTA has validated the test for seed vigour estimation for *Glycine max* based on the staining pattern (ISTA, 2022).

Seed must be imbibed prior to testing to initiate metabolic pathways of respiration. The intact imbibed seeds of 2 x 100 number are completely immersed in a 0.1% 2,3,5 triphenyl tetrazolium chloride solution in the dark for 3h at 35 ± 2^0C. Afterwards decant the solution and wash the seeds with water and kept them submerged in water during evaluation to avoid drying. Remove the seed coat with hand and cut the hypocotyl axis from the middle of cotyledons. Based on the staining patterns as described in the below figure (Fig. 1) the non-vigorous seeds can be identified.

The test requires experienced analyst to evaluate the results. Other living organisms such as fungi will also stain. The test also helps in determining viability of dormant seeds.

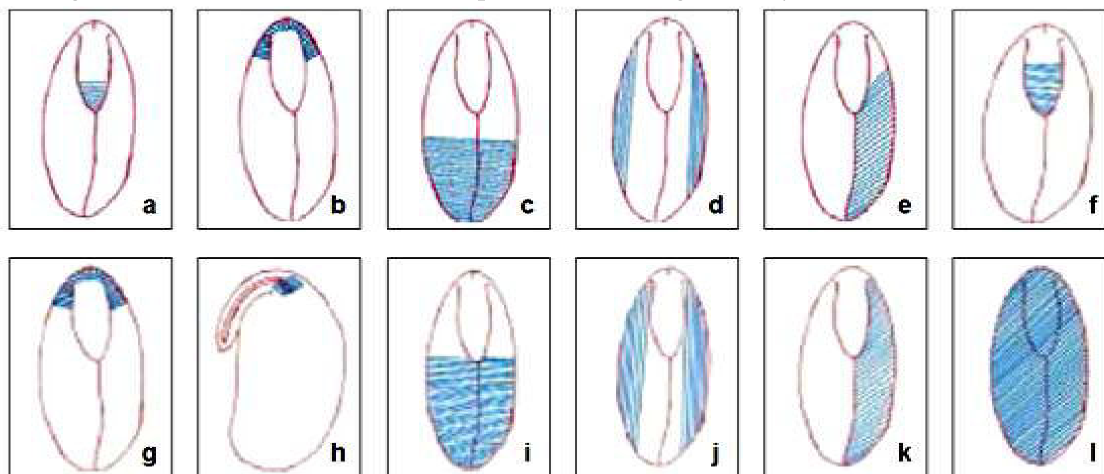


Fig.1: non-vigorous seeds, other staining. **a** Radicle with tissues up to ⅓ deteriorated, unstained or lost. **b** Joining area between embryo axis and cotyledons with deteriorated red tissues. **c** Cotyledons with tissues up to ½ deteriorated, unstained or lost. **d** Cotyledons with tissues up to ¼ deep deteriorated or unstained. **e** Cotyledon with tissues up to ¾ deteriorated, unstained or lost. **f** Radicle with more than ⅓ of deteriorated, unstained, or lost tissues. **g** Joining area embryo axis-cotyledons unstained. **h** plumule deteriorated or lost. **i**Cotyledons with more than ½ deteriorated, unstained or lost tissues. **j** Cotyledons with more than ¼ deep deterioration or unstained tissues. **k** Cotyledon with more than ¾ deteriorated, unstained or lost tissues. **l** Entire seed unstained.

3. **Respiration (R.Q.) test:** During the process of respiration, oxygen is taken up by seeds and CO_2 is released. The ratio of the volume of evolved per unit time to the volume of oxygen consumed per unit time is called Respiratory Quotient (RQ). The RQ were found to be more often related to the vigour than oxygen uptake alone. The rate of gas exchange is measured in the Warburg respirometer / apparatus.

4. **Conductivity test or Membrane Integrity Test:** The test was first recognized by Hibbard and Miller (1928) and it provides a measurement of electrolyte leakage from tissues. During seed deterioration membrane integrity is compromised and this causes leakage of electrolytes and solutes including sugars, amino acids etc. when immersed in water. Measurement of electrical conductivity of leachates provides an assessment of vigour. Seeds with low electrolyte leakage are high vigour seeds. ISTA has validated this test for five crop species viz., *Cicer arietinum, Glycine max, Phaseolus vulgaris, Pisum sativum* and *Raphanus sativus* (ISTA, 2022).

The moisture content of seeds is adjusted between 10 and 14 % before conducting the test. The seeds in four weighed replicates of 50 seeds each are to be used for this test. The seeds are soaked in 250 ml of distilled/ deionised water at 20 ^{0}C for 24 h. At the end of soak period gently swirl the leachate and measure the electrical conductivity (EC) using a pre-calibrated electrical conductivity meter. The EC was measured in $\mu S cm^{-1} g^{-1}$ of seed to the nearest 0.1 $\mu S\ cm^{-1}\ g^{-1}$. Lower the EC value higher is the vigour. The test results are influenced by initial moisture content, seed size and seed treatment.

Advantages of seed vigour tests

- ✓ A more sensitive index of seed quality than the standard germination test
- ✓ A consistent ranking of seed lots of acceptable germination in terms of their potential physiological and physical quality
- ✓ Information on emergence and storage potential of seed lots to plan marketing strategy
- ✓ Useful as quality control of seed production
- ✓ Useful in plant breeding programs

Limitations of seed vigour tests

- ✓ A precise referee testing method among seed laboratories is needed to arrive at a common vigour test methodology.
- ✓ Do not predict percentage field emergence,
- ✓ relative values are obtained from seed vigor tests
- ✓ Comparison of the results of different tests is difficult
- ✓ Experienced analyst required for interpretation of results

Exact points between acceptable and unacceptable levels of vigour have only been established for a few recommended tests (e.g. conductivity test for garden peas) and must be established for other frequently used vigour tests.

Conclusion:

To increase the yield it is essential to know the planting value especially under adverse and ever changing climatic conditions. The vigor tests give the valuable guidance about the storage potential and are useful for seed business, too. The ultimate user must be properly educated on the subject of seed vigour, before vigour test results can be effectively interpreted and understood. Till date very few tests that too for limited species have been validated by ISTA and there is a need to include a more reliable and reproducible tests on greater number of species at the earliest.

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Seed Analysis – Do's & Don'ts

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Introduction

Seed analysis involves evaluating all the quality attributes of seeds like physical purity, planting value of the seeds, moisture content, seed health, seed vigour, genetic purity etc., to offer quality seeds to farmer and to minimize the risk of planting low quality seeds. Seed analysis identifies seed quality problems and their probable causes, helps to determine labeling specification, establish quality and provide a basis for price and consumer discrimination among lots in the market and helps to identify the need for drying and processing of seeds. Use of quality seed is one of the strategies to increase production and productivity of any crop. International Seed Testing Association (ISTA), Association of Official Seed Analysts (AOSA) and The Society of Commercial Seed Technologists (SCST) involved in developing, adopting and publishing internationally agreed standard procedures (Rules) for sampling and testing seeds. It is important to follow the established seed testing rules while doing seed analysis to obtain uniform results all over the world.

Essential of Seed analysis

The assessment of seed quality is a highly skilled job. The important prerequisites to carry forward the seed analysis work would include the following.

i. Establishment of seed testing laboratory
ii. Seed analyst/ seed testing officer with scientific background to serve the laboratory
iii. Availability of the sets of seed testing procedures, rules and Manuals
iv. Seed Herbarium of the crop variety and weed seed species of the area for correct identification of admixtures present in the sample
v. Controlled seed storage rooms for storing the guard samples and samples awaiting the tests.

The seed testing laboratory must have functional autonomy in its working and should be headed by a person who had sufficient experience of seed analysis work. The Seed Testing Offices must be well acquainted with the procedures, rules and regulations. He must be well verse in the administrative and financial powers for effective and efficient functioning of the laboratory.

Historical events

1869- The 1stSeed Testing station was established in Thrandt, Saxony, Germany by Friedrich Nobbe
1876- Hand book of seed testing was published by Friedrich Nobbe
1871: E. Moller Holst, established a seed testing lab in Copenhagen, Denmark
1876 -1stSeed Testing station in US
1900- Europe 130 Seed Testing Stations
1924- International Seed Testing Association (ISTA)-to develop procedures and to promote uniform application
1939 -Association of official Seed Analysts (AOSA)

1960 -First Seed Testing Laboratory was established in India (CSTL at IARI, New Delhi)
1967 -First seed testing manual was published for uniform testing
2005-National Seed Research and Training Centre (NSRTC) was established
2007-National seed quality control laboratory which service as Central seed testing laboratory (CSTL) was established at NSRTC

Do's & Don'ts Seed Analysis

Seed sample handling

Seed sample handling needs more care to obtain accurate results in seed analysis. Seed testing capacity of laboratory is 50samples but the numbers of samples received are 100. Then the excess samples need to be kept in cold storage condition or the condition where the addition of Relative humidity in percentage and temperature in degree Fahrenheit should not exceeds 100. One should wear gloves when handling the treated seed sample (generally submitted by private seed companies). Proper procedure and equipment need to be utilized to draw working sample from submitted sample. The guard sample should be always being kept in cloth bag at cold storage condition (15°C and 50% RH) to avoid deterioration.

Purity analysis

The seed analyst should have enough knowledge on morphological characteristic of crop seeds. He should have description of pure seed sample. Seed analyst should gain enough knowledge on identification of weed seeds and other crop seeds. While doing seed analysis 50% rule should be strictly followed. If the seeds are 50% in size then it should be kept under inert matter. If the size of seeds more than 50%, than the seeds should be considered pure seeds irrespective of presence or absence of embryo. After purity analysis, when we add the weight of all the four components (Pure seed, other crop seed, weed seed and inert mater) it should be 100% not more than or less than 100 per cent. Generally percentage calculation has to be carried out for all the components other than pure seeds. Pure seed percentage should be calculated by deducting the value of other components from 100. If there is any deviation that is addition of all the components more than or less than the value of 100 then the deviating value should be adjusted in the pure seed value. Because, the increasing or decreasing of total components weight is mainly due to drying or moisture gaining of pure seed.

ODV test

ODV test results are mostly depends on the experience of the seed analyst. There is need of seed herbarium of important varieties of different crops. Having seed morphological character will help the analyst. Therefore it is important to compile the morphological characters of all the existing varieties.

Germination testing

Germination substrate plays a crucial role in realizing accurate test results. Sterilization of substrate is important before using it for germination test to avoid microbial contamination. We can go for dry sterilization in hot air oven at 130°C for 30 minutes. Infra-red sterilization chamber can be utilized to sterilize the germination paper

(sterilization period is 24hrs). Sand can be sterilized under sun after washing during summer. Germination substrate should be neutral in pH. The analyst must conduct Electrical conductivity (EC) and pH test of germination subtracted to ensure it's quality. To conduct the EC and pH, the germination paper should be soaked for one hour in double distilled water, after one hour decant the soaking solution and test the pH and EC. The EC should be <1 $dSmol^{-1}$ and pH should be 7-7.5 for good quality germination paper. To test the sand, soak 20g of sand in 40ml of double distilled water for half an hour and decant the solution after half an hour and go for EC and pH test. Here also EC should be <1 $dSmol^{-1}$ and pH should be 7-7.5 for good quality sand. Periodical calibration of EC and pH meter is required to ensure the accurate testing EC and pH of solution decanted from soaking of germination paper and sand. Onion seed germination in germination paper is conducted to know the toxicity of germination paper. Germination paper should be used only once. Sand can be used for germination test more once with cleaning and sterilization after conduction every germination test. If the sand is used for testing treated seeds, then the sand should be discarded after one time use. In germination test use butter paper only once to cover roll towel. Roll towel should not be covered fully and 2 inches of bottom portion of roll towel has to be left oven for aeration and excess water drainage. In paper of butter paper, polythene cover and be used more one time after sterilization before each test. Use double distilled water for germination test and even in germinator and for humidification of germination chamber is important to avoid microbial contamination. NSRTC has a distillation unit with the capacity of 50liters / hour distilled water production. Periodical disinfection of sample handling area, germination putting area, counting area, seed germinator and germination chamber and is very important to avoid cross contamination. The person entering inside the germination chamber should change his shoes and need to wear lab coat. The temperature of seed germinator and germination chamber should be cross checked by thermometer periodically. While putting germination, sterilization of hand after handling every sample is also necessary to avoid cross contamination. Wearing gloves at the time of putting and counting of treated seed is highly essential to avoid contact with toxic substances. Sodium hypochlorite 0.2-0.5% and 5% salt solution (brain solution) is use as surface disinfectant in seed testing laboratory. Germination counting area, germination putting area should not be the same to avoid cross contamination. Proper classification of seedling is essential while germination counting, to confirm the planting value of seeds. If the seedlings are showing primary infection, than that should be classified as abnormal seedlings. If the seedlings are showing secondary infection, than that should be considered as normal seedling. Care should be taken while counting hard seeds and fresh ingeminated seeds.TZ test can be conducted to ensure the viability of hard seeds (1% solution is used for dicot seeds and 0.5% solution is used for monocot seeds). Then we can go for dormancy breaking treatment. Here the treatment period should not be included with germination testing period. The germination test should be conducted as per the recommended procedure to get reliable and reproducible result. Some time there is a need to conduct seed vigour test. Specific procedure recommend for each crop should be followed with care.

Moisture testing

The sample must be received in 700 gauge polythene bag. The sample should be processed immediately after opening of the bag. Temperature should not be raised while grinding sample wherever is necessary. Moisture test can be conducted both by moisture meter and hot air oven. The estimated moisture values derived from both the methods should not differed more than 0.2%. Hot air oven method is accurate and recommended method. The moisture meter should be calibrated periodically for accurate moisture estimation of seed samples. Recommended temperature and duration should be followed for estimation of moisture content of each crop otherwise biochemical changes in the seed cause variation in estimated seed moisture value.

Seed health testing

The sample received for seed health testing should not be a treated one. Sterilization of blotter paper, Petri plate, media and equipment is always necessary to avoid secondary source of contamination. The analyst should acquire enough knowledge on identification of spore and pathogens. Precaution should be taken while keeping or removing the seed samples under near UV light. Wearing proper mask is recommended while examining the fungal pathogen. Periodical cleaning of microscope, laminar air flow chamber, seed health testing room and other equipment and tools is must to avoid cross contamination in seed health testing.

Genetic purity testing

The seed analyst needs to obtain genetically pure seed sample from the concern breeder or institute to raise the reference variety. Along with reference variety the varietal characteristic also need to be received from the concern breeder or institute. No thinning and rouging are recommended in grow out test. The suspected off type plant should be tagged and monitored during its entire growth period to confirm its off type nature. The person involved in grow out test should have enough knowledge about morphological characteristic of submitted varieties or hybrids. If GOT is of any variety / hybrids is conducted no growing area then there is a need to provide all the favourable condition by raising crop in protected condition for proper expression of all the varietal charters.

Conclusion

Tolerance table should be utilized wherever is necessary. Recommended pattern and procedure should be followed to report the test results of all the seed quality analysis. Need periodical training to seed analyst with respect recent advancement. Overall cleanliness of laboratory should be ensured for better seed testing environment and seed testing results.

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Importance and use of tolerances in seed testing

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The greatest hazard in agriculture is sowing seed that has not the capacity to produce an abundant crop of the required cultivar. Seed testing has been developed to minimize this risk by assessing the quality of seed before it is sown. The large scale movement of seed from one state to another leading to significant seed trade development taking advantage of the wide variety of climatic and consequently growing conditions has now brought appreciable awareness amongst farmers about quality of seeds. Because of the nature of seeds and their movement from one area to another, the result of tests need to be reproducible not only within a given laboratory but between laboratories, therefore great care needs to be exercised in the procedures used, in the correct use of equipment and in following the best judgment humanly possible in making various decisions in evaluations, necessary in seed testing. Seed being the living commodity, no two samples taken from the same seed bag or same seed lot are likely to be identical. However, if an entire seed lot could be tested, its true value would definitely be ascertained, and therefore this is neither feasible nor ordinarily possible. Thus in seed testing the quality of the lot must be determined from the sample representing the entire lot. Four major situations may arise which may continue to put pressure on seed testing laboratories to assure that their results are reproducible.

1. Testing of the same seed lot in different laboratories.
2. Seed lot testing under seed certification programme.
3. Seed lot testing for checking of seed certificate under seed law enforcement.
4. Statutory testing of seed lot for law enforcement to be used as evidence in law courts.

For the above situations and to ensure that analysis conducted within the laboratory is accurate, it in necessary at the part of the analyst to make sure that the result being reported is valid/reproducible. The application of an appropriate statistical method to test the results of seed testing enables the analyst to determine the validity of results within a calculated range of limits, the amount of this range in called the **'Tolerance'**. For seed quality determination, replicated test is conducted from same sample or different samples drawn from the same lot and replicated tests are conducted in one laboratory or different laboratories. It is generally observed that the test results may not be the same. The differences among the result are compared with non-significant permissible value supported with the statistical evidence or calculated range of limits. In seed testing, this maximum non-significant calculated range of limit or expected variation is called **"Tolerance Number"** and a series of such tolerance numbers is called **'Tolerance Table'**. Tolerance tables are used for testing the significance of the precise estimates for (a) to describe the seed quality (b) to decide if the estimate agrees well enough with another estimate or specification. In other words the object of using tolerances is to provide a means of assessing whether or not the variation within the test results or between the tests is sufficiently wide to raise doubt about the accuracy of results.

A. Sources of Variation in Test Results:

No two samples taken from the same seed bag or same seed lot are likely to be identical. The size of a lot varies depending upon the seed size. Experience world wide, has shown that variations do occur in sampling process, among replicates even when recommended sampling procedures are used. However experiences have shown that variations between the laboratories have been greater than that due to random sampling variation. There are five main sources of variation are recognized (a) bag to bag variation (b) in bag variation (c) working sample variation (d) between analyst variation and (e) in analyst variation.

B. Basic Assumptions for Using Tolerances:

There are three basic assumptions to use tolerances (a) the seed lot from which the sample is drawn should be relatively homogeneous (b) the sample must be drawn randomly as per seed sampling recommended procedure from container or locations in the lot (c) Bias must be avoided in conducting test.

C. Where to Apply Tolerances:

C.1. Within Seed Testing Laboratory:

Before release of seed analysis reports, a seed analyst has to make sure about the accuracy and reproducibility of results. Several situations normally arise in the seed-testing laboratory when tolerance should be applied to decide the validity of the tests. Some of them are:

(a) Germination test: To decide whether the replicate wise performance of a germination test is comparable or retest- is needed.

(b) Purity analysis: Comparing the replicate wise results of physical purity of different components and to decide whether the test conducted is valid or retest is necessary.

(c) Referee Test: To ensure that seed testing laboratories are achieving reproducible results, referee testing is arranged and tolerances are applied to evaluate the accuracy of their results.

C.2. For Certification:

(a) The Central Seed Committee (CSC), Government of India has prescribed the minimum seed certification standards for various crops. As a practice these standards are also taken as such for label information, even if the seed analysis results may be higher than the prescribed standards, the actual prescribed standards are only mentioned on the table.

(b) Competition in the seed trade based on difference in seed quality in future is very likely. Moreover, the farmers' awareness for seed quality measurements may necessitate labeling as per the actual seed analysis report. Thus label information can be well above the prescribed seed standards or at par with the standards. In either case seed is fit for certification.

(c) The seed standards are so formulated and prescribed that they ensure a reasonable level of quality for the seed user and that they can be achieved by a majority of seed producers *i.e.* it is neither kept high or very low. Therefore, for initial certification tolerances are not applied.

(d) According to the prevalent practice, certification is valid for a period of eight months. On expiry of this period the certification agency draws a fresh sample of the lot and sends it to the laboratory .On receipt of results decision on extending the validity period *i.e.* revalidation/ re-certification is taken. At present, revalidation is done only if the seed analysis results are at least meeting the prescribed seed standards without the application of tolerances. But the same situation is being viewed differently for the purpose of seed law enforcement for assessing the accuracy of label information tolerance tables are used.

(e) From the above point (d) following points become clear:

Average analysis of two half samples or two whole sample		Tolerance for differences between	
		Half working samples	**Whole working samples**
1	2	3	4
99.95-100.00	000-0.04	0.23	0.16
99 90-99 94	005-009	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	0.49-0.44	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	0.00-1.24	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
….*	….	….	….
….	….	….	….
….	….	….	….
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

(i) For seed law enforcement, tolerances are used and thus labels are retained even if the laboratory report is less than the label information but difference is within tolerances units; and

(ii) For revalidation/re-certification, tolerances are not used and thus lot is declared as unfit if the laboratory result is lower than the prescribed standards irrespective of whether the difference is within or out of tolerance.

C.3. Outside seed testing laboratory:

(a) Seed law enforcement- Label prescription:

Under seed law enforcement, the information given on the label affixed/attached on each container of the lot may be above the prescribed seed standards but under prevailing practice in India the information about seed quality attributes given on the label is the same as per prescribed seed standards for each seed quality attribute. In several other countries the seller normally sells seeds at higher price when label information is above the prescribed seed standards. In such situations the analysis results of seed samples drawn by seed inspectors are compared with the actual information given on the label and tolerances are applied to determine if label information is close enough to analysis results.

(b) Seed law enforcement -Seed standard prescription:

The central seed committee has prescribed crop wise minimum seed certification standards class wise for labeling seeds. At present the seed certification standards are as such taken for labeling, therefore information on the labels given as per prescribed standards even though the sample might be recording higher germination and purity than the standards. In other words for law enforcement, tolerances are applied whether or not the lot is at least equal to the prescribed minimum limit given on the label.

D. How to Use Tolerance Table:

Several tolerance tables (1 to12) have been developed and are available in the reference materials such as the International Seed Testing association's "Hand Book of Tolerances and Measures of Precision for Seed Testing" (1963) and International Seed Testing Rules (1999.) Separate tolerance tables are available for different situations. Depending upon the situation the tolerance table to be used is decided.

D.I. Use of Tolerance Table within and between laboratories Test Results

(a) How to Use For Purity Analysis Results:

To compute the tolerances of the components of purity analysis it is necessary to have the information about the magnitude of variation associated with each source of variation, which affects the percent, estimated on a sample from the seed lot.

Table 1: Tolerances for comparing duplicate working samples from the same submitted sample for any component of purity for either chaffy or non-chaffy seeds, at 0.05% probability.

*. …Indicates the other values in the series

Table –2: Tolerance for any component of Purity analysis between two laboratories, at 1% probability.

Average Analysis		**Tolerance**	
50-100%	**Less than50%**	**Non-Chaffy Seed**	**Chaffy Seed**
1	**2**	**3**	**4**
99.95-100.00	0.00-0.04	0.18	0.21
99.90-99.94	0.05-0.09	0.28	0.32
99.85-99.89	0.10-0.14	0.34	0.40
….*	….	….	….
90.00-90.99	9.00-9.99	2.48	2.92
88.00-89.99	10.00-11.99	2.65	3.11
….	….	….	….
72.00-73.99	26.00-27.99	3.76	4.44
70.00-71.99	28.00-29.99	3.84	4.51
65.00-69.99	30.00-34.99	3.97	4.66
60.00-64.99	35.00-39.99	4.10	4.82
50.00-59.99	40.00-49.99	4.21	4.95

*. …Indicates the other values in the series

Table-3: Tolerances to test whether 2 estimates of number of weed seeds or crop seeds are significantly different 0.05% Probability.

Av. Of 2 Est.	3	…	9-10	..	96-102	..	199-209	..	301-313	..	395-409
Max Tol Diff	5	…	9	..	28	..	40	..	49	..	56

***. …Indicates the other values in the series**

Examples of Use of Tolerance Table for Purity Analysis Results:

(i) To ensure accuracy in a laboratory, it is often desired that the submitted sample is divided first into working samples. Two different seed analysts then analyze each working sample. Suppose purity percentages of the whole working samples in two tests (by the two analysts) were **98.5** and **96.2**. The question thus arises whether their difference is acceptable. To determine, this add the two values (**98.5+96.2=194.7)** and calculate the mean (**194.7/ 2** = **97.35).** Now in column **1** of table **1** find the range that compares with the average figure; it is **97.25-97.49**. The tolerance value given in column **4** of the table is **1.20.** The difference between the two working sample is **98.5-96.2=2.3**. Since this difference is more than the tolerance the results are not equal, comparable or acceptable; hence' fresh test has to be conducted by drawing another working sample.

(ii) Sometimes samples of the same seed lot may be tested by two different laboratories. For e.g. one laboratory finds **98.5%** pure seed and the other finds **96.2%** pure seed. To find out whether these values are within tolerance and acceptable, calculate the average, refer table **2** and identify the range in which the average falls. This is **97.25-97.49** and the tolerance permitted is **1.39** for non-chaffy seed and **1.63** for chaffy seeds. The difference between the two laboratory results is **2.3**. Therefore it is out of tolerance for both chaffy and non-chaffy seeds and the analysis should be redone.

(iii) The examples elaborated in (i) above relate to purity analysis results reported on weight basis. Foreign seeds are also reported by number per unit weight. Tolerance values are determined as given above but using table 3 this table can be used *in* comparing the number of seeds of single species or the total of two or more species.

(b) How to Use For Germination Test Results:

The use of tolerances with respect to germination test apply to anyone of the following: (i) percent normal seedlings seeds (ii) per cent abnormal seedlings (iii) per cent dead seeds (iv) percent hard seeds or (v) the sum of any two or three of these four attributes. Germination tolerances should be computed allowing for random sampling variation. Among the many causes of significant differences between or among germination tests are (i) chance alone (ii) variation due to equipment and materials (iii) methodology (iv) errors or inconsistency in distinguishing between normal and abnormal seedlings (v) fungi or bacteria (vi) chemicals on the seed (viii) inaccurate counting (viii) non random selection of seeds for test or (ix) actual change in the per cent germination between tests.

Table-4: Maximum tolerated ranges in germination percent for deciding whether to retest; allowing for random sampling variation only.

Average percent germination		**No. replicates of 100 seeds**		
		4 rep.	**3 rep.**	**2 rep.**
1	**2**	**3**	**4**	**5**
99	2	5	4	-
…	…	…	…	…

97	4	7	6	5
…	…	…	…	…
93-95	7-8	10	9	8
…	…	…	…	…
78-80	21-23	16	15	13
77	24	17	15	13
73-76	25-28	17	16	14
…	…	…	…	…
64-66	35-37	19	18	15
…	…	…	…	…
51-55	46-50	20	18	14

*. …Indicates the other values in the series

Tabel-5 Tolerance for deciding whether germination tests conducted on same sample are compatible; allowing for random sampling variation only.

Average percent Germination		**Tolerance**
More than 50 Percent	50 Percent or less than 50 %	
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	42-50	8

*. …Indicates the other values in the series

Table-6 Tolerance for comparing tests between laboratories for germination percent.

Average Percent		**Tolerance**
More than 50 Percent	50 Percent or less than 50 %	
1	**2**	**3**
99	2	2
97 To 98	3 To 4	3
94 To 96	5 To 7	4
91 To 93	8 To 10	5
87 To 90	11 To 14	6
82 To 86	15 To 19	7
76 To 81	20 To 25	8
70 To 75	26 To 31	9
60 To 69	32 To 41	10

51 To 59	42 To 50	11

*. …Indicates the other values in the series

Examples of Use of Tolerance Table for Germination Test Results:

(i) The germination test in a seed-testing laboratory is always conducted as replicated tests of 100 seeds each. The numbers of replications are normally four; for official evaluations such as in seed law enforcement work, the replications may be three; and for service sample it may be two. For working out the mean it is necessary that results of the replicated analysis are reliable and within the acceptable tolerance limits. For example, if the four replications showed germination percentage (Normal seedlings) as- **90, 92, 93** and **83**; the average worked out to be the **90** percent. In table **4** column **1**, locate the range in which the average percent germination value of **90** falls. It is **(89-90**). The maximum tolerance permitted amongst replicates in column **3**, is **12**. The maximum difference between these replicates is **93-85=8**. Thus the difference between the replicates is within the tolerance limits and therefore the results of the test are acceptable for adding and working out the average.

(ii) Tolerance table-**5** is used when two series of replicated tests are made on a sample; for example, for a seed lot the first test replicates averages **85%** and the second test replicate averages **89%.** The average of these two independent tests would be **87%,** while the difference between the average of the two series of tests is **89-85=4%.** In table-**5**, column **1**, the average percent germination of **87%** falls in the range **85-90%.** The tolerance permitted here is **5**, the difference between the average of the two series of tests is only **4**; the results are hence within tolerance and therefore, the average of two tests can be reported. If the difference would have been otherwise then one more test should be made.

(iii) Table-**6** is used when the germination test results are compared between two laboratories. The details of the use of the Table are the same as given in (ii) above.

E. Use of Tolerances under Seed Law Enforcement Programme:

The above-mentioned six different tolerance tables are used for different situations. The basic principle in the use of these tables is that they are used only when the results reported by the laboratory show lower germination or pure seed or higher inert matter, other crop seed or weed seed content than what is given on the label. But, during certification, labeling is done only when the laboratory results are in conformity with the prescribed seed standards. Thus on the label either the actual results or the prescribed seed standards may be printed.

Under seed law enforcement two different situations may thus arise; (i) Comparing laboratory's results with the details on labels; and (ii) Comparing laboratory's results with the prescribed minimum seed standards indicated on the table. Specific tolerance tables for individual situations are, therefore, to be referred.

(a) Table no.-7 is meant for comparing the laboratory's results of purity test with the details on label. To use this table both the laboratories results and the details on label should be in percentage by weight.
(b) Table no.-8 is used to compare laboratory's results on other crop seed and weed seed by number per unit weight with the details on label. To use this table both the laboratory's results and details on label should be in number per unit weight.
(c) Table no.- 9 is for comparing germination results reported by the laboratory with the details on table.
(d) Table no.-10 and 11 are meant for comparing laboratory's purity analysis results with the minimum standards prescribed.
(e) Table no.- 12 is to be used for comparing laboratory's germination results with the minimum seed standards prescribed.

The use of table no. 7 to 12 is elaborated by taking the following example

Attributes	**Percentage on the table**	**Prescribed Seed (Percentage)**	**Percentage Reported by the laboratory**
Pure Seed (Minimum)	99.0	98.0	97.0
Inert matter (maximum)	1.8	2.0	1.1
Other crop seed (Maximum)	0.1	0.1	0.1
Weed Seed (Maximum)	0.1	0.1	Trace
Germination (Minimum)	94	85	80

(f) From the above (a to e), two points emerge:
(i) Whether the labeling information provided is valid or not even after applying tolerances,
(ii) If even after applying the tolerances the information given on the label does not come in conformity,

The Seed Inspector may demand change of the label provided it is in conformity to the prescribed seed standards; in the event of nonconformity the seed inspector may ask for removal of the label and tag and then the lot cannot be sold as labeled seed.

For the use of all these tables **Hand Book of Tolerances and Measures of Precision for Seed Testing** can be referred. The summery of the use of these tables is given below:

Summary			
Situation for using tolerance	**Table number**		
	Purity components by weight	Foreign seed count	Germination
a) Within the laboratory	1	3	4-5
b) Between two laboratories	2	3	6
c) Seed law enforcement comparing laboratory results with the label information	7	8	9

d) Comparing laboratory results with the prescribed seed standards	10	11	12

(e)**Comparing the laboratory results with the label information:-** (i) Table- 7 is meant for any purity component is percentage by weight. The following steps are involved in verifying whether or not the difference between laboratory result and the details on the label is within tolerance.

(f) Work out the average of laboratory results and the percentage on label :

Pure Seed: 99.0 + 97.0 = 196 Average=196/ 2 = 98.0

(i) The difference between the laboratory result and the percentage indicated on the label is (99.0-97.0)=2.0. This difference is higher than the tolerance level and therefore, labeling is wrong.

(ii)If the level of weed seed or other crop seeds are given in number on the label, Table 8 can be used to determine the accuracy of labeling for seed law enforcement. If the information given on the label compares satisfactorily with the number found upon testing labeling *is* regarded as acceptable.

(iii) Table-9 is used for comparing the laboratory result with the information given on label for germination. The procedure is:

(iv) Calculate the average of the two *viz.* laboratory germination and the information on label; i.e. : 80+94/2 = 87

(v)Fit the average suitably in Table 9, Column A & B as the case may be and find the tolerance level against this. The tolerance given in column C against the average *viz.* 87 is 6.

(vi)The difference between laboratory result and the percentage indicated on label is 94-80=14. This is higher than the tolerance level and therefore labeling is wrong.

(g) Comparing the laboratory result with the minimum standards prescribed :

(i) Table-10 is meant for any purity component in percentage by weight. The procedure is: Work out the difference between the laboratory result and the standard prescribed; for example, pure seed.

Laboratory result:97.0 Standard : 98.0 Difference : 1.0

Tolerance level in column C, Table 10 against 98% is : 0.61 .The difference between the laboratory result and the standard in more than the tolerance level; hence lot is not fit to be sold as standard seed.

(ii) The same procedure is followed to compare the information on other crop seeds reported by the laboratory in number per unit weight. Table 11 is referred for this purpose.

(iii) For germination Table 12 is used. The difference between the laboratory germination and the standard prescribed in worked out. For example, it is (85-80)=5. Taking the number of replicates as four of 100 seeds each the total number of seeds in 400. Fit the standard prescribed *viz.* 85% suitably in Table-12 column C. The tolerance is 6 while the actual difference is only 5. Thus the laboratory result is in conformity with seed standards and seed lot can be soled as standard seed.

(g)The same seed lot was found to be wrongly labeled when the comparison was made between the information given on the label and the laboratory result.

Summary			
Situation for using tolerance	Table number		
	Purity components by weight	Foreign seed count	Germinatio n
a) Within the laboratory	1	3	4-5
b) Between two laboratories	2	3	6
c) Seed law enforcement comparing laboratory results with the label information	7	8	9
d) Comparing laboratory results with the prescribed seed standards	10	11	12

Table- 7 Tolerances for comparing the results of a purity test with the label information (when both the laboratory results and label information are in percentage by weight)

Average of 2 estimates		Non Chaffy Seeds	Chaffy Seeds
A	B	C	D
99.95-100.00	0.00-0.04	0.12	0.14
…..	…..	…..	…..
99.50-99.54	0.45-0.49	0.44	0.52
99.40-99.49	0.50-0.59	0.47	0.56
…..	…..	…..	…..
99.00-99.09	0.90-0.99	0.61	0.72
98.75-98.99	0.00-1.24	0.66	0.78
…..	…..	…..	…..
97.00-97.24	2.75-2.99	1.02	1.21
96.50-96.99	3.00-3.49	1.08	1.28
…..	…..	…..	…..
95.00-95.49	4.50-4.99	1.28	1.51
94.00-94.99	5.00-5.99	1.37	1.62
…..	…..	…..	…..
90.00-90.99	9.00-9.99	1.76	2.07
88.00-89.99	10.00-11.99	1.88	2.20
…..	…..	…..	…..
70.00-71.99	28.00-29.99	2.72	3.20
65.00-96.99	30.00-34.99	2.81	3.30
60.00-64.99	35.00-39.99	2.90	3.41
50.00-59.99	40.00-49.99	2.98	3.50

Table 8: Tolerances for comparing the foreign seed numbers found in laboratory test with label information (when both the laboratory result and label information are given in number per unit weight).

A	0.00	0.05	0.10	0.15	..	0.80	0.85	..	1.00	2.00	3.00
B	2	2	3	4	..	4	5	..	5	7	9
A	4.00	5.00	6.00	7.00	8.00	9.00	10.0	11.0	12.0	13.0	14.0
B	10	12	13	15	16	18	19	20	22	23	24
A	15.0	16.0	17.0	18.0	19.0	..	40	41	42	..	500
B	26	27	28	30	31	..	56	58	59	..	554

A= Average of laboratory's result and label information. **B=** Tolerance Level

Hand book of Tolerances and Measures of Precision for seed Testing, Table F2, only 5% Probability column is used.

Table 9: Tolerances for comparing the laboratory germination result with label information

Average percent Germination		4Tests	3Tests	2Tests
A	B	C	D	E
99	2	2	2	1
98	3	3	2	2
97	4	3	3	2
96	5	3	3	3
95	6	4	3	3
94	7	4	4	3
93	8	4	4	4
92	9	5	5	4
91	10	5	4	3
90	11	5	5	4
89	12	5	5	4
88	13	6	5	4
87	14	6	5	4
86	15	6	6	5
..	..	..	..	..
78	23	7	7	6
..	..	..	..	..
75	26	8	7	6
67	34	8	8	6
..	..	..	..	..
63	38	8	8	6
62	39	9	8	6

60	41	9	8	6
59	42	9	8	7
..	..	..	..	..
51	50	9	8	7

Based on 5% probability
Hand book of Tolerances and Measures of Precision for seed Testing, Table G2,

Table -10 Tolerance for comparing laboratory results of purity analysis with the standard prescribed (when both the laboratory result and the standard are in percentage by weight)

Specification %		**Non Chaffy seed**	**Chaffy seed**
A	**B**	**C**	**D**
99.95-100.00	0.00-0.04	0.10	0.11
..	..	..	..
99.50-99.54	0.45-0.49	0.32	0.38
99.40-99.49	0.50-0.59	0.34	0.41
..	..	..	..
99.00-99.09	0.90-0.99	0.44	0.52
98.75-98.99	1.00-1.24	0.58	0.57
..	..	..	..
97.00-97.24	2.75-2.79	0.73	0.86
96.50-96.99	3.00-3.49	0.77	0.91
..	..	..	..
95.00-95.49	4.50-4.99	0.96	1.07
94.00-94.99	5.00-5.99	1.97	1.15
..	..	..	..
90.00-90.99	9.00-9.99	1.24	1.46
88.00-89.99	10.00-11.99	1.33	1.56
..	..	..	..
70.00-71.99	28.00-29.99	1.92	2.26
65.00-69.99	30.00-34.99	1.99	2.33
60.00-64.99	35.00-39.99	2.05	2.41
50.00-59.99	40.00-49.99	2.11	2.48

Only the 5% probability column is used.
Hand book of Tolerances and Measures of Precision for seed Testing, Table P15.

Table 11: Tolerances for comparing the foreign seed numbers with a specified standard(when both the laboratory result and standard are in number per unit weight).

A	0.00	0.05	0.10	0.15	..	0.80	0.85	..	1.00	2.00	3.00
B	1	1	2	2	..	3	4	..	4	6	7

A	4.00	5.00	6.00	7.00	8.00	9.00	10.0	11.0	12.0	13.0	14.0
B	9	10	11	13	14	14	16	18	19	20	21
A	15.0	16.0	17.0	18.0	19.0	..	40	41	42	..	500
B	23	24	25	26	27	..	52	53	53	..	538

A= Standard **B= Rejected**

5% Probability level is shown.
Hand book of Tolerances and Measures of Precision for seed Testing, Table F, only

Table 12: Tolerances for comparing the laboratory result of germination test with a specified minimum limit of germination standard.

Average percent Germination		4Tests	3Tests	2Tests
A	**B**	**C**	**D**	**E**
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	3	6
..	..	..	..	..
86	15	5	3	7
..	..	..	..	..
82	19	5	4	7
81	20	5	4	8
..	..	..	..	..
79	22	6	4	8
..	..	..	..	..
75	26	6	4	9
..	..	..	..	..
70	31	7	5	9
69	32	7	5	10
..	..	..	..	..
59	42	7	5	11
..	..	..	..	..
57	44	8	5	11
..	..	..	..	..
51	50	8	5	11

Only 5% Probability column is used.
Hand book of Tolerances and Measures of Precision for seed Testing, Table G-7,

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Varietal Purity Testing through Biochemical Methods

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Seed is a key input in all crop production. All cultural practices are designed to exploit the full genetic potential of the seed sown. No agricultural practices (for example tillage, cultivation, weeding, fertilizer, pest and disease control) can increase crop yields beyond the limit set by the seed quality. Seed is therefore the baseline for success or failure of the crop planted. To achieve high yields, one must plant high quality seed. ***Seed** quality* describes the potential performance of a seed lot. Trueness to variety; the presence of inert matter, seed of other crops, or weed seed; germination percentage; vigor; appearance; and freedom from disease are important aspects of seed quality. Thus, quality seed insures good germination, rapid emergence, and vigorous growth. These aspects translate to a good stand (whether greenhouse or field). Among these attributes, the genetic purity governs the maximum yield potential that could be realized from using a particular cultivar.

Variety is defined as group of plants having clear distinguished characters which when reproduced either sexually or asexually retains these characters and thus the term 'varietal purity/genetic purity' generally infers that plant population of a particular variety is homogenous and genetically identical i.e. true to type with respect to the cultivar it is claimed for.

To maintain cultivar trueness and purity of seed, Seed certification schemes, controlled pedigree systems and rules and regulations for seed growing and distribution are in place. However, there are still possibilities of contamination or loss of purity during seed multiplication like

(1) Natural crossing with another cultivar especially in open pollinated crops,
(2) Mutation,
(3) Unclean harvesting equipment,
(4) Carelessness at the processing plant and
(5) Mistakes in bagging and tagging.

Thus, in order to realize the full potential of the cultivar, the genetic purity becomes imperative. Genetic purity test is done to verify any deviation from genuineness of the variety during its multiplications. For certification, genetic purity test is compulsory for all foundation and certified hybrid seeds. Moreover, the determination is valid only if the species or cultivar is stated by the sender of the sample and if an authentic standard sample of the species or cultivar is available for comparison. The characters compared may be morphological, physiological, cytological or chemical. The traditional Grow Out Test (GOT) to determine the seed genetic purity test based on morphological markers is time consuming and dependent on the environment. To overcome this disadvantage, biochemical markers are being used in many crops.

Chemical Tests:

With increasing number of varieties and limited diversity for morphological characters, it is difficult to establish the identity and distinctness of a variety and instead can be categorized into different groups. This is more so when newer varieties are developed using germplasm with limited level of genetic diversity or when convergent selection towards similar morphology is practiced. For such situations, inclusions of additional biochemical markers are often found useful. It is well known that "all inherent morphological manifestation of

Variety differences must ultimately have a biochemical difference but not all biochemical differences are necessarily reflected morphologically". With biochemical differences being more numerous than morphological differences, each truly distinct cultivar should posses' correspondingly distinct biochemical characteristics. This confirms the broad scope of utilization of biochemical tests in variety identification and purity testing. The bio-chemical tests recommended by ISTA and AOSA are given below:

ISTA	AOSA
• Phenol test for wheat • Lugol's test for lupin • Electrophoretic technique for wheat, oat, peas, lolium, maize and sunflower	• NaOH test for wheat • Copper sulfate-ammonia test for sweet clover • HCl test for oat • Peroxidase test for soybean • KOH test for rice and sorghum

Application of such tests like phenol colour reaction, peroxidase test and electrophoresis techniques for the said purpose has been discussed below.

1. ***Phenol Test for Wheat:*** The phenol test has been used for many years for determining varietal purity in wheat.

   Principle: Phenol test assesses tyrosinase (also known as polyphenoloxidase or catecholase) activity in seeds and outer glumes by simple colour reaction. Tyrosinase enzyme present in the seed coat oxidizes the phenol vapours, using atmospheric oxygen, to produce brown colour. The intensity of brown colour developed (dark brown, brown, light brown and no colour development) (Fig. 1) depends on the quantity of enzyme, which is a variety characteristic. This variety varietal difference with respect to quantity of tyrosinase enzyme present in seed coat/outer glumes has been exploited for variety characterization, identification and purity testing through phenol colour reaction test in many crops viz., wheat, oat, pearl millet, rice, maize, etc. International Seed Testing Association (ISTA) has recommended this test for ensuring the genuineness of wheat cultivars

   Procedure: For conducting this test seeds are soaked in water for 16 hr at 20°c. The soaked seeds are then placed in petridish lined with filter paper soaked with 1% phenol solution and kept at 30°c. The reaction is noted after 4 hours. The intensity of colour varies from black, dark brown, brown and light brown. Based on the colour developed on the seed coat the varieties may be grouped.

Like any other characteristic, phenol colour reaction alone is not sufficient to establish distinctness of the variety and could be used as one of the diagnostic characteristic for establishing the identity of a variety. As a diagnostic characteristic, it is widely used for ensuring the genetic purity of seed lots of wheat. In many cases phenol test may also be utilized for providing the answer regarding the level of admixture. If phenol colour reaction is not uniform as that of authentic sample then it is confirmed that the sample contains admixtures and is not true to the type.

2. ***Sodium Hydroxide Test for Wheat:*** Often it is difficult to distinguish red and white cultivars of wheat especially if a sample has been treated with a fungicide as those usually incorporate an orange or pink dye or difficulties may also be experienced if the sample has been subjected to weathering in the field during harvest, or premature ripening has resulted in a large proportion of grains with vitreous, as opposed to starchy, endospermThe method consists of immersing each four replicates of 25 grains in 25ml. of 5% sodium hydroxide solution in a 10cm. glass Petri dish, and noting the color changes over a period of one hour. The grains of both red and white varieties assume a bright yellow color immediately when they are immersed in the solution of sodium hydroxide. But after 10 minutes the grains of the red varities become dark orange which deepens to an orange-brown color after 60 minutes. By contrast, the initial bright yellow color of the white grains fades to a straw yellow color after 10 minutes in sodium hydroxide solution, and then shows little further change.

3. ***KOH test for Sorghum*** : The presence or absence of a darkly pigmented testa or undercoat layer can he used to differentiate sorghum cultivars. The dark pigment in the testa has been identified as tannic acid. Seeds with brown seed coats and the seed with white seed coats should be soaked for 5-10 min respectively in the KOH-bleach solution. The mixture should be stirred gently periodically. Then the seed should be rinsed with tap water and dried on paper towel. It should be observed for dark pigmented tannic acid and light pigmented testa.
4. ***Peroxidase Test for Soybean:*** Seed coat of soybean cultivars response to this test. After removing the seed coat, seeds are treated with 10 drops of 0.5% guaiacol followed by hydrogen peroxide solution. Peroxidase positive (high peroxidase activity) seeds are indicated by a reddish-brown solution and peroxidase negative (low peroxidase activity) indicated by a colourless solution in the test tube.

5. ***Lugol's test for lupin:*** The lugol test has been used for many years for determining sweet (low-alkaloid) variety in *albus lupin*. The cotyledons after imbibation are soaked in lugols solution for 30 sec and rinsed with water therafter. The presence reddish brown colour indicateds high alkaloid variety while sweet variety does not develop any colour.

Electrophoresis method

The electrophoresis technique is increasingly utilized as an additional approach for varietal identification and characterization based on storage protein. The uniformity of seed protein profiles is additive in nature and not much influenced by the environmental conditions makes it a unique and powerful tool for genotype identification. Intrinsic changes in the plant such as chromosomal rearrangements or even doubling of the

chromosome numbers leave no or very small effect on the seed proteins profile. They are also independent of cultivar morphology and physiology, hence proteins can be regarded as markers for the structural genes. The analysis of protein composition can be considered to be an analysis of gene expression and can be used as an ideal means of varietal discrimination. It is a rapid and relatively cheap method and eliminates the plants to grow upto maturity. ISTA published the first protocol for variety testing using polyacrylamide gel electrophoresis (PAGE) for wheat and barley in 1987. Since then the technique has been refined and modified suitably for a number of species viz., *Triticum*, *Hordeum*, *Lolium*, *Pisum*, *Zea*, *Helianthus*, *Avena* etc. Work is underway to examine application of these for several other crops by the ISTA Working Group on Variety Testing.

Gel electrophoresis is used in testing plants/crops amongst other things. The results are analyzed to determine genetic purity. The benefit of electrophoresis is that it gives strong genotypic data for crops. In this technique, proteins/Isoenzymes are separated into distinct bands in a support medium of polyacrylamide or starch gel under the influence of electric current applied across the medium. The separation is due to the differences in the size/charge/both of the protein/isoenzyme involved. The difference between the varieties is established based on presence or absence of a particular protein/isozyme band at a particular position in a support medium, which is marked by Relative mobility (Rm) value of that particular band.

Electrophoresis may be defined as the migration of charged ions in an electric field. Electrophoresis: Differential movement or migration of charged molecules (ions) in solution through a matrix which acts as a molecular sieve in an electric field. The separation of molecules is according to their size, shape and/or charge. Negatively charged molecules (anions) will be attracted towards anode while positively charged molecules (cations) will move towards cathode.

Rate of migration depends on:

1. Molecular charge (net charge)
2. Molecular shape and size
3. Strength of the electrical field,
4. Ionic strength, viscosity, and temperature of the medium.

Proteins being the direct gene products reflect the genomic composition of varieties. Gel electrophoresis of proteins and isozoenzymes is, thus, a powerful tool to distinguish between genotypes of plant species (Wrigley and Bates, 1999).

To understand the process of electrophoresis refreshing the basic knowledge of protein structures will be useful.

The basic unit of protein molecules is an amino acid, having the general formula of:

Polypeptides are formed by amide linkages (peptide bonds) between the carboxyl group of one amino acid and amino group of the next, eliminating a H_2O molecule.

Proteins are macromolecules and have four different levels of structure – primary, secondary, tertiary and quaternary.

Primary structure: A protein's primary structure is the unique sequence of amino acids in each polypeptide chain that makes up the protein. It is just a list of which amino acids appear in which order in a polypeptide chain, not really a structure. But, because the final protein structure ultimately depends on this sequence, this was called the primary structure of the polypeptide chain.

Secondary structure: A protein's secondary structure is whatever regular structures arise from interactions between neighboring or near-by amino acids as the polypeptide starts to fold into its functional three-dimensional form. Secondary structures arise as H bonds form between local groups of amino acids in a region of the polypeptide chain. Rarely does a single secondary structure extend throughout the polypeptide chain. It is usually just in a section of the chain. The most common forms of secondary structure are the α-helix and β-pleated sheet structures and they play an important structural role in most globular and fibrous proteins.

Tertiary Structure: The tertiary structure of a polypeptide chain is its overall three-dimensional shape, once all the secondary structure elements have folded together among each other. Interactions between polar, nonpolar, acidic, and basic R group within the polypeptide chain create the complex three-dimensional tertiary structure of a protein. When protein folding takes place in the aqueous environment of the body, the hydrophobic R groups of nonpolar amino acids mostly lie in the interior of the protein, while the hydrophilic R groups lie mostly on the outside. Cysteine side chains form disulfide linkages in the presence of oxygen, the only covalent bond forming during protein folding. All of these interactions, weak and strong, determine the final three-dimensional shape of the protein. When a protein loses its three-dimensional shape, it will no longer be functional.

Quaternary Structure: The quaternary structure of a protein is how its subunits are oriented and arranged with respect to one another. As a result, quaternary structure only applies to multi-subunit proteins; that is, proteins made from one than one polypeptide chain. Proteins made from a single polypeptide will not have a quaternary structure.

In 1924, T. B. Osborne classified the seed storage proteins into a four groups based on their solubility in a series of solvents

Albumin	:	Soluble in water. Mostly enzymes
Globulin	:	Soluble in dilute salt solutions. Mostly storage bodies
Prolamins	:	Soluble in aqueous alcoholic solutions. Storage proteins
Glutenins	:	Soluble in dilute acidic or alkaline solutions. Mainly, structural proteins

Electrophoretic methods recommended by ISTA (1999) for various crops species

Crop species	**Method**
Hordeum (barley)	*Acid polyacrylamide gel electrophoresis* (PAGE) of alcohol-soluble proteins (hordeins)

Triticum (wheat)	*Acetic acid urea polyacrylamide gel electrophoresis (A-PAGE) of alcohol-soluble proteins (gliadins) at pH 3.2*
Triticum and × Triticosecale (wheat and triticosecale)	*Sodium Dodecyl Sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using 100* individual seeds. . If a comparison is being made with a standard value, sequential testing using batches of 50 seeds can be undertaken in order to minimize the workload. A simple check on the identity of a single major constituent of a seed lot can be done using less than 50 seeds.
Pisum and Lolium	*SDS-PAGE of seed proteins using 100* individual seeds are used in *Pisum.* Very precise estimates of varietal purity may require a larger sample. If a comparison is made with a standard value, sequential testing using batches of 50 seeds can be undertaken in order to minimize the workload. A simple check on the identity of a single major constituent of a seed lot can be done using less than 50 seeds. *while in Lolium bulked* seeds are analysed. In *Lolium the method will serve to* verify seed lots but will not permit the detection of admixtures.
Zea mays	*Alcohol soluble proteins (Zeins) extracted from individual* seeds are separated by UTLIEF. Normally 200 single seeds analysed. For reports and issue of ISTA certificate, 400 single seeds are analysed.
Avena sativa	*Urea/ethylene glycol-soluble proteins (Avenins) are* extracted from seeds and separated by Acid-PGE

Seed protein electrophoresis:

Native PAGE: ***Principle:*** In native PAGE, proteins are separated according to the net charge, size, and shape of their native structure. Electrophoretic migration occurs because most proteins carry a net negative charge in alkaline running buffers. The higher the negative charge density (more charges per molecule mass), the faster a protein will tend to migrate. At the same time, the frictional force of the gel matrix creates a sieving effect, regulating the movement of proteins according to their size and three-dimensional shape. Small proteins face only a small frictional force, while larger proteins face a larger frictional force. Thus, native PAGE separates proteins based upon both their charge, mass and structure. Because no denaturants are used in native PAGE, subunit interactions within a multimeric protein are generally retained and information can be gained about the quaternary structure.

The polyacrylamide gel is formed by the vinyl polymerization of acryl amide monomers into long polyacrylamide chains and cross linking these by the inclusion of an bifunctional monomer N, N' methylene-bis-acrylamide (bis). The polymerization reaction produces random chains of polyacrylamide incorporating a small proportion of bis molecules which, in turn, react with groups in other chains forming cross-links that results in a three dimensional network. The concentration of acryl amide determines the polymer chain length, while the concentration of bis determines the extent of cross-linking. Thus, the gel density, elasticity, mechanical strength and pore size are determined by the concentration of acryl amide and bis used.

The pore size of the gels is greatly influenced by the acryl amide concentration. The effective pore size decrease with the increasing acryl amide concentration. The total acryl

amide + bis concentration in a gel mixture is represented by % T while the concentration of the cross-linking monomer (ie. Bis) is represented by % C. The pore size increases with the increase in % C. Gels ranging from 3 to 30% acryl amide concentration can be made and used for separating molecules of size up to 1 x 10^6 Daltons. Gels with linear gradients of increasing acryl amide concentration are also used for a greater resolution, particularly when analyzing a mixture of proteins of very low to very high molecular weights.

To initiate the process of polymerization of acryl amide, a catalyst, such as ammonium persulphate or riboflavin is added along with an accelerator e.g. N, N, N'N'-tetra methylenediamine (TEMED). TEMED catalyzes the formation of free radicals from persulphate, while these radicals initiate the polymerization reactions. In a riboflavin - TEMED system, on the other hand, though the photo-oxidation of riboflavin can produce free radicals necessary for polymerization, TEMED is also added to ensure complete and uniform polymerization.

Selection of a suitable gel and buffer system

The mixture of proteins to be analyzed by PAGE consists of molecules differing in size and net charge. The degree of separation of these proteins is greatly influenced by different conditions of pH, concentrations of acryl amide and bis, ionic strength, potential gradient, strength of the electric field (i.e. current/volts applied), running duration, temperature etc. Selection of optimum conditions depends on the type of the sample to the examined. Thus, for a sample of proteins with high molecular weights, a large pore gel (low acryl amide concentration) will be suitable. However, when the separation is based mainly on charge difference, gels with large pores are used even for smaller molecules of proteins.

The mobility of a protein molecule increases as the pH of the buffer is farther from its isoelectric point, therefore, a pH should be chosen that maximizes mobility differences among the components of protein mixture. As a general rule, basic proteins are better separated at an acidic pH, while most of the proteins having iso-electric points within pH 4.0 are best separated under alkaline condition of pH 8-9. In common practice, PAGE is carried out at a pH range of 3 to 10.

The ionic strength of the buffer system should be such that the sample is kept in solution with adequate buffering capacity. The electrical resistance is lower at higher ionic concentrations of the buffer, which results in higher current and greater heat generation, though the bands are generally sharper (if the heat can be dissipated) at higher concentrations of buffer. Generally, buffers within the range of 0.05 to 0.1 M are found most suitable, though lower or higher ionic strengths can also be used.

SDS-PAGE: ***Principle:*** SDS is an anionic detergent. Samples to be run on SDS–PAGE are firstly boiled for 5 min in sample buffer containing b-mercaptoethanol and SDS. The mercaptoethanol reduces any disulphide bridges present that are holding together the protein tertiary structure, and the SDS binds strongly to, and denatures, the protein. Each protein in the mixture is therefore fully denatured by this treatment and opens up into a rod-shaped structure with a series of negatively charged SDS molecules along the polypeptide chain. On average, one SDS molecule binds for every two amino acid

residues. The original native charge on the molecule is therefore completely swamped by the negatively charged SDS molecules. The rod-like structure remains, as any rotation that tends to fold up the protein chain would result in repulsion between negative charges on different parts of the protein chain, returning the conformation back to the rod shape. The sample buffer also contains an ionisable tracking dye, usually bromophenol blue, that allows the Electrophoretic run to be monitored, and sucrose or glycerol, which gives the sample solution density thus allowing the sample to settle easily through the electrophoresis buffer to the bottom when injected into the loading well. Once the samples are all loaded, a current is passed through the gel. The samples to be separated are not in fact loaded directly into the main separating gel but to a shorter (approximately 0.8 cm) stacking gel is poured on top of the separating gel. In stacking gel , the protein concentrate in a very tight band between glycinate and Cl boundaries. In separting gel, The negatively charged protein–SDS complexes now continue to move towards the anode, and, because they have the same charge per unit length, they travel into the separating gel under the applied electric field with the same mobility. However, as they pass through the separating gel the proteins separate, owing to the molecular sieving properties of the gel. Quite simply, the smaller the protein the more easily it can pass through the pores of the gel, whereas large proteins are successively retarded by frictional resistance due to the sieving effect of the gels.

Isoelectric focusing: Principle : Isoelectric focusing (IEF) is an Electrophoretic method for the separation of proteins, according to their Isoelectric points (pI), in a stabilized pH gradient. The method involves casting a layer of polyacrylamide gel containing a mixture of carrier ampholytes (low-molecular-weight synthetic polyamino-polycarboxylic acids). When an electric field is applied to .such a gel, the carrier ampholytes arrange themselves in order of increasing pI from the anode to the cathode. Each carrier ampholyte maintains a local pH corresponding to its pI and thus a uniform pH gradient is created across the gel. If a protein sample is applied to the surface of the gel, it will also migrate under the influence of the electric field until it reaches the region of the gradient where the pH corresponds to its Isoelectric point. At this pH, the protein will have no net charge and will therefore become stationary, or "focused" at this point. Proteins are therefore separated according to their charge, and not size as with SDS gel electrophoresis.

A modification of IEF on polyacrylamide gel with a thickness of 0.15 mm is called **ultrathinlayer isoelectric focusing (UTLIEF)** and offers a faster, safer and cheaper technique for protein separation. This technique offers a faster, safer, and cheaper way to verify varieties using the separation of storage protein in the seed. Isoelectric focusing is the separation of amphoteric substances (carrier ampholytes). The ampholytes migrate along the pH-gradient under the influence of electrical forces between electrodes until they arrive at their isoelectric point, where the net external electrical charge is zero and they become immobilized. At the isoelectric point, storage protein can be seen on an electrophoretogram of the polyacrylamide gel.

Two-Dimensional electrophoresis: Principle: The first dimention is to separate proteins by their native charge using IEF. All proteins have an inherent natural charge due to the

side chains of their amino acid residues. The total number of positively and negatively charged amino acids determines the natural charge on a protein. For separation by charge, the sample is loaded into the top of a gel with a pH gradient. When an electric field is applied, the proteins move along the pH gradient until their charge is neutralized. This step is usually done on a tube-shaped gel. After this step is run, the gel is removed from its tube. The second dimension of 2D-PAGE is separation by size. The tube gel containing the separated proteins is treated with SDS to denature the proteins and coat them with negative charges as in regular PAGE. The tube gel is laid along the top of a polyacrylamide slab gel, and the proteins are separated by size

Standard reference method of ISTA (2019) for the verification of varieties of *Hordeum* (barley) by Polyacrylamide Gel Electrophoresis (PAGE)

Principle

The alcohol-soluble proteins (hordeins from *Hordeum*) are extracted from seeds and separated by PAGE at pH 3.2. The pattern of protein bands produced (electrophoregram) is related to genetic constitution and can be considered as a 'fingerprint' of a variety. The 'fingerprints' can be used to identify unknown samples and mixtures, by single seed analysis.

As a guideline, it is recommended that 100 seeds are used. Very precise estimates of varietal purity may require a larger sample. If a comparison is being made with a standard value, sequential testing using batches of 50 seeds can be undertaken in order to minimize the workload. A sample check on the identity of a single major constitution of a seed lot can be done using less than 50 seeds.

Apparatus and equipment

Any suitable vertical electrophoresis system should be used.

Chemicals

All chemicals should be of 'Analytical Reagent' grade or better

a. Acrylamide ('specially purified for electrophoresis')
b. Bisacrylamide ('specially purified for electrophoresis')
c. Urea
d. Glycial acetic acid
e. Glycine
f. Ferrous sulphate
g. Ascorbic acid
h. Hydrogen peroxide (or ammonium persulphate and TEMED)
i. Monothioglycerol (or 2-mercaptoethanol)
j. Pyronine G (or methylgreen)
k. Trichloroacetic acid
l. Ethanol
m. 2-chloroethanol
n. PAGE Blue G-90 (or PAGE Blue 83) (or any reagent equivalent to the 'Coomassie Blue' series of dyes).

Solutions

a) Extraction solution: pyronine G (or methyl green) (0.05%) in 2-chloroethanol (20%) containing urea (18%) and monothioglycerol (or 2-mercaptoethanol) (1%) (keep cold or prepare fresh)
b) Stock tank buffer solution : glacial acetic acid (4 ml) and glycine (0.4 g), made up to 1L with water; keep cold
c) Stock gel buffer solution: glacial acetic acid (20 ml) and glycine (1.0 g), made up to 1L with water; keep cold.
d) Staining solutions:

I. Trichloroacetic acid (100 g) in 1 L of water
II. PAGE Blue G-90 (or PAGE Blue 83) 1 g) in ethanol (100 ml).

Procedure

Single seeds are crushed and transferred to 1.5 ml polypropylene centrifuge tubes. Extraction solution (0.3 ml) is added, the contents of the tubes are thoroughly mixed and the tubes are allowed to stand overnight at room temperature. The tubes are centrifuged at 13,000 x g and the supernatants used for electrophoresis. Extracts can normally be stored at 4^0C for 3-4 days.

Gel preparation

Clean and dry gel cassettes are assembled, according to the design of the equipment. Treating the glass plates with silicon prior to assembly can facilitate subsequent removal of the gel. The gel cassettes can incorporate a plastic backing sheet (e.g. 'Gel Bond PAG', FMC Corporation). This supports the gel during subsequent operations. To make 100 ml of gel medium, stock gel buffer (approx. 60 ml) is taken and the following added-acrylamide (10 g), bisacrylamide (0.4 g), urea (6 g), ascorbic acid (0.1 g), ferrous sulphate (0.00 5 g). The solution is stirred and made up to 100 ml with stock gel buffer solution. Freshly prepared 0.6% hydrogen peroxide solution (0.35 ml per 100 ml of gel medium) is added, mixed quickly and the gel poured. Note that the gel mixture can be cooled to near freezing prior to the addition of the peroxide. Polymerisation should be complete in 5-10 minutes. An acrylic 'comb' is placed in the top of the cassette, to make wells in the gel. The gel mixture should over fill the cassette, or be overlaid with water, to ensure satisfactory polymerization of the upper surface.

Note that as an alternative to the hydrogen peroxide catalyst, it is possible to use ammonium persulphate (0.1 ml of 10% solution, freshly prepared) and TEMED (0.3 ml) added to the gel mixture prior to pouring the gel.

Electrophoresis

The acrylic comb is removed from the gel and the sample wells washed with tank buffer. The tank is filled with an appropriate volume of buffer (depending on the equipment used). Samples (10-20 µl) are loaded into the wells and the gel placed in the tank, ensuring that the sample wells are completely filled. Electrophoresis is carried out at 500 V (constant voltage) for twice the time taken for the pyronine G maker dye to leave the gel, or three times if methyl green is used as a tracking dye. Water should be circulated through the buffer tank to maintain the temperature at 15-20^0C.

Fixing and staining

The gel cassette is removed from the tank, opened and the gel placed in a plastic box containing 5- 10 ml of 1% PAGE G90 (or PAGE Blue 83) in 200 ml of 10% trichloroacetic acid. Staining is complete in 1-2 days and de-staining is not usually needed. Precipitated stain should be scraped from the surface of the gel. The gel is washed in water to enhance the stain and can then be examined or photographed. Any blue background in the gel is removed by washing in 10% trichloroacetic acid. Gels can be stored in polythene bags at 4^0C for many months without deterioration.

Nomenclature of Gliadin and Hordein bands

Gliadin and hordein bands can be identified either by measuring their relative mobilities (Wrigley, C.W., Autran, J.C. and Bushuk, W., 1982, Advances in Cereal Science and Technology 5, 211-259), by means of an electrophoretic formula (Konarev, V.B., Gavrilyuk, I.P., Gubareva, N.K.and Peneva, T.I., 1979, Cereal Chemistry 56, 272-278) or by designation of patterns (Shewry, P.R., Pratt, H.M., Faulks, A.J., Parmar, S. and Miflin, B.J., 1979, Journal of the National Institute of Agricultural Botany, 15, 5-40.

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Seed Germination Testing

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A germination test determines the maximum germination potential or viability of the seed. Germination is an important parameter while determining the seed quality. Moreover, this is a statutory requirement for seed certification and marketing for labeling and seed law enforcement. Thus the ultimate aim of testing the germination in seed testing laboratory is to obtain information about the field planting value of the seed sample and by inference the quality of seed lot. The results also assist in comparing performance potential or superiority of the different seed lots.

In order for germination to occur, three conditions must be fulfilled. First, the seed must be viable; that is, the embryo must be alive and capable of germination. Second, internal conditions within the seed must be favourable for germination i.e. any physical or chemical barriers to germination must have disappeared. Third, the seed must be subjected to favourable environmental conditions, the essential factors being available water, proper temperature, a supply of oxygen and sometimes light. Although in any one seed each of these conditions may have an effect distinct from the others, the beginning of germination may be more often determined by the interactions among them.

Definition and principle of evaluating germination test:

Germination represents a dynamic period in the life cycle of plants as a seed makes the transition from a metabolically quiescent to an active and growing entity. In general, germination is transformation of the embryo into seedling. It is defined as the emergence and development from the seed embryo of those essential structures which, for the kind of seed in question, indicate its ability to produce a normal plant under favourable conditions. The essential structures include root system, shoot axis (hypocotyl, epicotyl, mesocotyl), coleoptile and cotyledons (ISTA, 1985). Seedlings with essential structures are considered as normal seedling while, seedlings devoid of an essential structure viz., showing weak or unbalanced development; decay or damage affecting the normal development of seedling are not considered in calculating the germination percentage.

Essential equipments and supplies for germination test

The following equipments and supplies are essential to carry forward the germination tests in the seed testing laboratories.

1. **Seed germinator:** The seed germinators are the essential requirement for germination testing for maintaining the specific conditions of temperature, relative humidity and light. The seed germinators are generally of two types, namely: Cabinet germinator and walk in germinator. The cabinet seed germinators are essential under the situations, where various kinds of seeds that require different sets of conditions, are being handled in the laboratory. The number of the pieces of the germinators required by the laboratory will depend on the

number of seed samples and the species being analysed by the laboratory. The seed testing laboratories that handle large number of seed samples and require maintaining only fewer (2-3) sets of temperature conditions, the walk-in-germinators are preferred. Such germinators are more useful for conducting the germination tests in sand media, which require large germination space.

2. **Counting devices:** The counting devices include the counting boards, automatic seed counter and vacuum seed counter. These devices are required to aid germination testing by minimizing the time spent on planning the seeds as well as to provide proper spacing of the seed on germination substrata. Counting boards are suitable for medium and bold sized seeds, while vacuum counter can be, used for small sized seeds. In the absence of counting devices, the work may be accomplished manually.

3. **Other equipments:** The other equipments required for germination testing include the refrigerators, scarifier, hot water bath, incubator, forceps, spatula, germination, boxes, plastic plates, roll- towel stands and plastic or surgical trays, etc. A large oven with temp. Range 100 -200 C is also required for sterilizing the sand.

4. **Miscellaneous supplies, glassware and chemicals:** Germination paper (Creppe Kraft paper or towel paper, sunlit filter paper and blotters) and sand are the basic supplies required for germination tests. In addition, the laboratory may also require some glassware, such as Petri dishes, beakers, funnel, measuring cylinders, muslin cloth, rubber bands and tubes etc. and certain chemicals like Potassium nitrate, Thiourea, Gibrellic acid, and Tetrazolium chloride for specific purposes. Voltage stabilizers are required for the supply of the constant electric current. The voltage stabilizers are essential for costly germinators, air-conditioners and refrigerators. Under the situations of erratic power supplies and breakdowns, electricity generators are also required.

Care of equipments: The seed analyst must ensure that:
1. All the equipments are in proper working condition
2. The germinators are maintaining correct temperature
3. The relative humidity inside the germinator is maintained 90--98%
4. The phytosanitary conditions of the germinators and germination trolleys are adequate
5. The germinators are disinfected periodically by flushing with hot water; solution of Potassium permanganate or chlorine water
6. The temperature and the R.H. of the walk-in-germinators are recorded daily and displayed on a chart
7. The floor, ceiling and walls of the walk-in-germinator are devoid of cracks, crevices;
8. Evenly plastered and duly painted to avoid contamination by fungus, bacteria or insects.

Substratum (Media) for germination

Seeds require certain conditions for germination. The most important requirements are substrata (media), moisture, temperature and light. Suitable substrata for seed germination include paper towels, blotter paper, filter paper, cotton, vermiculite, sand or soil. The

accuracy and reproducibility of the test is very much dependent on the quality of substrata being used. The substrata must meet the following qualities:

- It must be easy to handle and use.
- It must provide adequate aeration and moisture to the germinating seedlings.
- It must be non-toxic to germinating seedlings.
- It must be free from moulds and other microorganisms.
- It should make good colour contrast of the substrate for judging seedlings.
- It must be less expensive

A. Germination Paper : The most widely used substrate are filter paper, blotter and Kraft paper towel (creped). Paper media are easy to handle, cheap and occupy less space. The paper should be made up of cotton or other purified cellulose. The fiber content of the paper should be 100 per cent chemically bleached wood cellulose. The strength of paper should be uniform throughout the area and should resist tearing when handled during test. The germination paper should have good capillarity rise and should have the following quality characters. In case of filter paper, Whatman 60 No. filter paper discs are generally used. It is not re-usable.

Specifications for paper substrate

- **Composition:** The fiber content of the paper should be 100 % chemically bleached wood, cotton or other purified vegetable cellulose with an ash content of 1.5 % by mass.
- **Texture:** It should be open and porous in nature. The roots of the seedlings should grow on the paper and not into it.
- **Strength**: It should have sufficient strength to enable it to resist tearing when handled during the test. It should have mass of 95-100 m/m^2 and a bursting strength of 2kg/ cm^2.
- **Moisture capacity:** The paper should have the capacity to hold sufficient water for the whole of the test period.
- **pH :** The pH should be between 6.0 – 7.5
- **Storage:** It should posses the ability to be stored for long period without losing its texture or the qualities mentioned above.
- **Sterilization**: Upon purchase it must be sterile and also be amenable for sterilization in oven or pressure cooker without losing its qualities mentioned above. It should also be free from pathogens.
- **Free from toxic chemicals**: The paper media is tested using sensitive species like *Phleum, Agrostis, Festuca, Brassica or Allium* sps. The seeds may be placed on two layers of germination paper in box and watered. After 3 days for mustard and 6 days for onion seedlings are observed. If the paper is non-toxic the seedling growth is normal if toxic, abnormalities like stunted root with discoloured root tip will be noticed. The root hairs will be bunched and plumules will be shortened.
- **Determination of capillary rise**: Ten strips of germination paper each 10 mm wide are cut with 5 strips along one direction and 5 in the opposite direction and immersed upto 20 mm of distilled water at 27 $\pm 2^0$C. After 5 min the water level is measured. A minimum raise of 15 cm must be observed (i.e. 3 cm / min).

- **Colour** : White or coloured with dyes that are non toxic. Generally white, blue or khaki coloured paper is preferred.

B. Sand Media: Sand should be reasonably of uniform size and free from very small and large particles. A particle size which passes through a sieve having holes of 0.8 mm diameter and be retained on a sieve having holes of 0.05 mm diameter is ideal. The sand should be free from foreign materials and pathogens. The sand should be capable of holding adequate moisture to provide continuous supply of moisture to the germinating seeds with pH range of 6.0 to 7.5. Its phyto-toxicity has to be checked before its use. Both river sand and quartz sand are used for evaluation of germination. It is a reusable media. It may need washing and sterilization before it is used. Never store the sand in the stores where fertilizers and chemicals are stored. If the sand is found to be heavily contaminated or changed in colour, after repeated use, it should be replaced with fresh stocks.

C. Vermiculite: For highly sensitive species vermiculite is used as substrata.

TEST CONDITIONS

1. Moisture: The moisture requirements of the seed will vary according to its kind. Large seeded species require more water than the small seeded species. It is essential that the substratum must be kept moist throughout the germination period. Care need to be taken that the sub-stratum should not be, too moist. The excessive moisture will restrict the aeration and may cause the rottening of the seedlings or development of watery seedlings. Except under the situations where a high moisture level is recommended (e.g. paddy and jute), the substratum should not be so wet that a film of water forms around the seeds. In situations, where low level of moisture is recommended (e.g. cucurbit seeds), the moist substratum should be pressed against the dry blotters or towel paper to remove excess moisture.

The water used for moistening the substratum must be free from organic and inorganic impurities. Normally the tap water is used. However, it is essential to measure the pH of water before its use. The pH of the water should be in the range of 6.5-7.5 (neutral). Under the situations where pH of the water is not satisfactory, distilled water or deionized water may be used. Under such situation care need to be exercised to aerate the tests frequently to provide oxygen supply to the germinating seedlings because oxygen level in distilled water is very low. The initial quantity of water to be added to the substratum will also depend on its nature and dimensions and also on the size and species of the seed to be tested. Subsequent watering, if any may be left to the discretion of the analyst but it should be avoided as far as possible because it may cause the variation in germination results. In order to reduce the need for additional watering during the germination period, the relative humidity of the air surrounding the seeds should be kept at 90-95% to prevent loss of water by evaporation.

2. Temperature: Temperature requirement varies with the species and with the age of seeds. At very low or high temperatures, the germination is prevented. The temperature should be as uniform as possible throughout the germinator and the germination process. Care should be taken that the temperature of tests does not exceed the prescribed level and

variation not more than $\pm$ 1^0C. Most of the agricultural crop species germinate between the temperature of 5°C and 35°C. Hence, required temperature should be provided with appropriate temperature control mechanism as per ISTA recommendation (Table 1).

According to the Rules for seed testing, either constant temperature or alternating temperatures are used. In constant temperature, a specific temperature is maintained during the entire test period and wherever, an alternating temperatures are prescribed, the lower temperature should be maintained for 16 hrs and the higher for 8 hours. A gradual change change-over lasting three hours is usually satisfactory for non-dormant seeds. However, a sharp change-over lasting 1 hour or less, or transfer of test to another germinator at lower temperature, may be necessary for seeds which are likely to be dormant. If temperatures cannot be conveniently altered over week-ends or holidays, the tests must be kept at the lower temperature. The daily alterations of temperature either brought out manually by transferring the test from one germinator to another or by changing the temperature of the chamber (Automatic seed germinator).

3. Light: Seeds of most of the species will germinate either in light or in darkness. However, illumination of the substrate from artificial source or by daylight is generally recommended during germination, for better seedling development to avoid etiolating and also to detect seedlings having chlorophyll deficiency. Seeds of tobacco and lettuce need light for germination. Cool tube lights or CFT are preferred to incandescent bulbs. Tube light emit more radiation in the normal sunlight range, while bulb emit more in IR range and hence is not preferred. Light intensity normally required for different crop seeds is 750 -1250 lux for atleast 8 hours in every 24 hours cycle. Under the situation where testing of the seed is required to be undertaken at alternating temperatures together with light, the test should be illuminated during high temperature period.

4. Air: Most seeds required aeration for higher germination. Some of the leguminous tree seeds exhale toxic fumes upon germination. Such seeds must be aerated to reduce auto-toxicity. Special measures for aeration are not usually necessary in case of top of paper (TP) tests. However, in case of 'Roll towel' tests (BP) care should be taken that the rolls should be loose enough to allow the presence of sufficient air around the seeds. In case of sand media, the sand should not be compressed while covering the seeds.

PROTOCOL FOR GERMINATION TEST

1. Drawl of Working Sample

The working sample for germination test consists of 400 seeds randomly selected either manually or with the help of counting devices from the pure seed fraction obtained from the purity test. A minimum of four replications of hundred seeds each or eight replications of fifty seeds or 16 replication of 25 seeds may be kept. The seeds for germination test must be drawn as follows in accordance with the following two situations:

Situation I: Both purity and germination tests are required,

- Seeds for germination test will be selected randomly from pure seed fraction received after conducting purity test.

- The counting of seeds must be made without discrimination as to the size and appearance.

Situation II: Only germination tests is required

1. If the percentage of pure seed is estimated to be 98 %, then pure seeds for germination test shall be taken indiscriminately from a representative portion of the submitted sample.
2. If pure seed is found to be less than 98 %, the seeds for germination test must be obtained by separating the sample into two components, namely (a) the pure seed and seeds of other species and inert matter. For this purpose, atleast one-fourth of the quantity required for regular purity analysis must be used after proper mixing and dividing the submitted sample. The seeds should not be pre-treated except those approved for improving the germination. If any pre-treatment is done then a mention must be made in the germination test result.

2. Conducting germination test

Germination test is always carried out with seeds counted randomly from the pure seed fraction. Testing of 400 seeds is recommended for all seed control and seed certification samples. However, at least 200 seeds may be tested for service samples. The seeds are counted and evenly spaced on the substratum by hand or by a vacuum counter or by a counting board. Some seeds that are fresh from harvest possess dormancy. When test seeds have dormancy, mere storage will reduce the dormancy. However some seeds possess dormancy even a month after harvest due to physical, physiological reasons and combination of both. Under such circumstances several methods have been prescribed by ISTA as provided below.

3. Pre-treatments for germination (Special treatments for breaking dormancy)

After the completion of germination period, if fresh ungerminated or hard seeds are observed in large proportions, a retest may be carried out either after a period of dry storage or by applying one of the special treatment for breaking dormancy as under.

A)Temperature treatment

a)Pre-heating : Warming seeds at 30-35^{0}C for 3 hrs or soaking in warm water (50^{0}C) for few hrs.

b) Pre-chilling : Seeds are kept in moist substratum at 5-10^{0}C for seven days before they are removed and shifted to the temperature prescribed for that crop species (Table 1.). In some cases even prolonged pre-chilling or re-chilling is recommended. The pre-chilling period is not included in the germination test period but the duration and temperature should be reported in the analysis certificate.

c) Pre-drying: Seed samples are heated at a temperature not exceeding 40^{0}C with free air circulation for period of upto seven days before placing for germination. Some time the pre-drying period can be extended.

d) Low temperature: Either low temperature or low temperature alternating with high temperature is provided. The germination may be slower and the test period can,

therefore, be extended by an additional period equivalent to that given in Table 1. Both temperature and duration should be mentioned.

B. Chemical treatment:

a) **Potassium nitrate (KNO_3):** Germination substratum is moistened with 0.2 % Potassium nitrate solution by dissolving 2 g in 1 liter of water. If necessary, subsequent moistening should be done with water.

b) **GA_3 :** The substratum is moistened with 500 ppm, GA_3, which can be prepared by dissolving 500 mg of GA_3 in one liter of water. If dormancy is weak then 200ppm solution is sufficient. If stronger, even 1000 ppm solution may be necessary. The time taken for breaking dormancy is not counted into germination period.

c) **Pre-washing:** When germination is affected by a naturally occurring water soluble substance in the seeds, which acts as an inhibitor, it may be removed by soaking and washing seeds in running water. After the preparation of seeds they have to be sown on the selected substrata according to the method prescribed below.

5. Sowing of Seeds in Media

A. Paper method

a) Top of the paper (TP): Seeds which are small and photoblastic are tested in top-of-paper method. In this method, place 2-3 layers of filter paper in petridish and moisten with enough paper. Remove excess water. Seeds are placed on a moist blotter paper or germination paper on petri dish. Seeds which germinate under dark (skotoblastic) are placed in between the two layers of blotter paper in petri dish.

b) Between paper (BP): The seeds are germinated in between layers of filter paper. This is done in two ways namely 1) Seeds are placed in between layers of filter paper in a plastic box and placed in germinator and seeds are placed in roll towel method.

c) Roll towel method: In this method, soak the germination paper in water and remove the extra moisture by pressing. Spread the sheet on a flat table and then seeds are placed on a germination paper in equal distance and covered with another strip of germination paper. To avoid evaporation of moist from the paper, a polythene sheet or butter paper is used to cover the germination paper. Keep a label with test number at one corner. Then the germination paper is rolled carefully and the entire assembly is kept in a germinator or partly immersed in water upright position (if germinator facility is not present). The disadvantage in this method is that daily observation without disturbance is not possible. Sometime the seeds germinate on the paper and the root penetrates the paper which causes difficulty during evaluation This method is done in case of seeds that are large and where seedling characters are to be observed and for those seeds which do not need light.

d) Pleated paper (PP) : Seeds are placed in pleated strips. The paper may have 5-10 pleats which can be made in the laboratory. Each pleat may have ten seeds. The pleated strips are kept in moistened bread boxes to ensure uniform moisture conditions. This

method may be used in TP and BP methods. This method is highly useful in calculation of speed of germination, where daily emergence of seedlings is counted

e) Inclined plate method: Seeds are placed over a strip of germination paper which is placed on a plastic or glass or acrylic plate. Then the seeds are covered with another paper and a polythene sheet is covered over it to prevent evaporation of moisture. The entire assembly is placed in 45 degree angle in a water tub/germinator.

B. Sand method

The seeds can be placed in two methods.

a) On sand (OS): The seeds are pressed into the surface of sand. This method is used for small and tiny seed (eg. *casuarina*), which may fail to germinate if sown even at little depths.

b) In sand (IS): The depth of sand bed should be approximately two inches. The seeds are placed on a leveled layer of moist sand in uniform spacing (not less than twice the length of the seed) and covered with 10-20 mm (approx. ¼ " to 1/2") of uncompressed sand depending on the size of the seed. To ensure good aeration it is recommended that the bottom layer of sand be loosened by raking before sowing. Put the cover on the germination boxes and place them under prescribed controlled temperature conditions.

5. Duration of the test

Each kind of seed based on their genetic potential are kept under the germination room condition for certain period as per ISTA which is noted as the germination/test period. Special dormancy breaking period (like chilling duration) is not included in the test duration.

The seeds placed for germination test are evaluated for germination after the germination period. First and second counts are usually taken with paper tests; however, only a single final count is made with sand test. At first and second counts, the seedlings which fulfill normal seedling conditions are removed, counted and discarded. All hard seed, diseased and abnormal seedlings, non germinated seeds are left until the final count when their number is recorded. Diseased seeds and seedlings which may affect healthy seeds may be removed before the final count. Hence, seedlings may have to be removed and counted at frequent intervals during prescribed period of the test when a sample contains seeds infected with fungi or bacteria.

If at the end of the prescribed test period some seeds have just started to germinate, the test period may be extended for an additional period up to 7 days. A test may be terminated prior to the prescribed time when the analyst is satisfied that the maximum germination of the sample has been obtained. The time for the final count is approximate and a deviation of 1-3 days is permitted. The first count may be delayed to permit the development of root hairs in order to be certain that the root development is normal, or may be omitted. Intermediate counts may be made at the discretion of the analyst to remove seedlings, which have reached a sufficient stage of development for evaluation, to prevent them becoming entangled. But the number of intermediate counts should be kept to minimum to reduce the risk of damaging any seedlings which are not sufficiently developed.

Table 1. Duration and specifications for conducting germination test as per ISTA

Crop	Botanical Name	Prescription for				Additional directions including recommendation for breaking dormancy
		Substrata	Temp (^{0}C)	First count (days)	Final count (days)	
FIELD CROPS						
CEREALS						
Barley	*Hordeum vulgare*	BP; S	20	4	7	Preheat (30-35^{0}C), prechill, GA_3
Paddy	*Oryza sativa*	BP; TP; S	20-30; 25	5	14	Preheat (50^{0}C) soak in water or KNO_3 24 hrs
Triticale	*Triticosecale*	BP	15-20	-	7	GA_3, Prechill
Wheat	*Triticum spp*	TP; BP; S	20	4	8	Preheat
MILLETS						
Barnyard Millet	*Echinocloa frumentacea*	TP	20-30	4	10	Prechill, KNO_3, GA_3
Finger Millet	*Elusine coracane*	TP; BP	20-30	4	8	0.2% KNO_3 (2-3 hrs)
Kodo Millet	*Paspalum scorbiculatum*	TP	20-30	7	20	KNO_3
Pearl Millet (Bajra)	*Pennisetum typhoides*	TP; BP	20-30	3	7	0.2% KNO_3 (2-3 hrs)
Sorghum	*Sorghum bicolor*	TP; BP	20-30;25	4	10	Prechill
PULSES						
Common vetch	*Vicia satva*	BP; S	20	5	14	Prechill
Lentil	*Lens culinaris*	BP; S	20	5	10	Prechill
OILSEEDS						
Groundnut	*Arachis hypogea*	BP; S	20-30;25	5	10	Remove shells, Preheat -40^{0}C
Linseed	*Lininum usitatssimum*	TP; BP	20-30;20	3	7	Prechill
Mustard	*Brassica*	TP	20-30;20	5	7	Prechill,

	juncea					KNO_3
Mustard (Black)	*Brassica nigra*	TP	20-30;20	5	10	Prechill, KNO_3
Niger (Ramtil)	*Guizota abyssinica*	TP	20-30	-	14	Prechill
Sunflower	*Helianthus anuus*	BP:S	20-30-25;25	4	10	Ethrel (25 ppm) 48 hrs
FIBRE CROPS						
Cotton	*Gossypum spp.*	BP;S	20, 30:25	4	12	Hot water (85^0C-1 minute)
FORAGE CROPS						
Bird wood grass (Dhama)	*Cenchrus setigerus*	TP	20-35	3	14	Preheat (40^0C)
Buffel grass	*Cenchrus cilliaris*	TP;S	20-35	7	28	Preheat ; Prechill, KNO_3
Burmuda grass (Doob)	*Cynodon dactylon*	TP	20-35	7	21	Prechill, KNO_3; Light
Dharaf grass	*Andropogan montanus*	TP	20-35	7	28	Prechill at 5^0C for two weeks
Dinanath grass	*Pannisetum pedicellatum*	TP	35;20-35	7	28	H_2SO_4 fro 5 min
Guinea grass	*Panicum maximum*	TP	15-35;20-30	10	28	Prechill, KNO_3
Indian clover (Senji)	*Melilotus indica*	TP;BP	20	4	7	Prechill
Lucerne	*Medicago sativa*	TP;BP	20	4	10	Prechill
Marvel grass	*Dichanthium anulatum*	TP	20-30	7	21	KNO_3
Oat	*Avena sativa*	BP;S	20	5	10	Preheat 30-35^0C, Prechill
Rye	*Secale cereale*	TP;BP;S	20	4	7	Prechill ;GA_3
Rye grass	*Lolium parenne*	TP	20-30	5	14	Prechill ; KNO_3
Sataria grass (Nandi	*Setaria anceps*	TP	20-35	7	21	KNO_3

grass)						
Stylo	*Stylosanthus spp*	TP	20-35	4	10	H_2SO_4
Sudan grass	*Sorghum sudanense*	TP;BP	20-30	4	10	Prechill
Teosinte	*Euchlaena mexicana*	BP;S	20-30;25	-	7	GA_3 1000 ppm – 24 hrs
GREEN MANURE AND MISCELLANEOUS CROPS						
Dhainch	*Sesbania sp*	TP;BP	20-30	5	7	Rub seed coat on sand paper
Indigo	*Indigofera hirsuta*	BP	20-30	-	14	Continue test for a further 5 days if hard seeds have begun to imbibe
Chicory	*Cichorium intybus*	TP	20-30;20	5	14	KNO_3
Garden cress	*Lepidium sativum*	TP	20-30;20	4	10	Prechill
Lotus	*Lotus corniculatum*	TP;BP	20-30;20	4	12	Prechill
Poppy (Opium)	*Papaver somniferum*	TP;	20	5	10	Prechill
Purslane	*Portulaca oleracea*	TP;BP	20-30	5	14	Prechill
Sugarbeet	*Beta vulgaris*	TP;BP;S	20-30 ; 15-25	4	14	Prewash multigerm 2 hrs ; monogerm 4 hrs
Tobacco	*Nicotiana tabacum*	TP	20-30	7	16	KNO_3
CUCURBITS						
Ashgourd	*Benincase hispida*	S	30-35	5	14	Light
Pointed gourd	*Trichosanthus dioica*	S	30-35	-	14	Dark, GA_3 500 ppm 24 hrs, Remove seed coat
Snakegourd	*Trichosanthus anguina*	S	30-35	-	14	Dark, GA_3 500 ppm 24 hrs, Remove seed coat

FRUIT VEGETABLES						
Chilli	*Capccum spp*	TP ; BP	20-30	7	14	KNO_3
Tomato	*Lycopercicum esculentum*	TP ; BP	20-30	5	14	KNO_3
BULB AND TUBER CROPS						
Leek	*Allium porrum*	TP ; BP	20-15	6	14	Prechill
Lesser yam	*Dioscora spp*	S	30	-	21	Prechill – 5^0C 3 day light
Onion	*Allium cepa*	TP ; BP	20-15	6	21	Prechill
True Potato Seed	*Solanum tuberosum*	TP	20-30	-	14	GA_3 500 ppm, 24 hrs; light
GREEN / LEAFY VEGETABLES						
Amaranth	*Amaranthus spp*	TP	20-30	-	8	Light
Lettuce	*Lactuca sativa*	TP;BP	20	4	7	Prechill
Parsnip	*Pastinaca sativa*	BP;TP;S	20-30	-	28	Prechill 5^0C
Spinach	*Spinaca oleracea*	TP; BP	15-10	7	21	Prechill
Spinach beet	*Beta vulgaris*	TP ; BP	20-30, 15-25	4	14	Prewash (multigerm 2 hrs ; genetic monogerm 4 hrs)
ROOT CROPS						
Celeriac	*Apium graveolens*	TP	20-30	10	21	Prechill, KNO_3
Garden beet	*Beta vulgaris*	TP; BP; S	20-30	4	14	Prewash multigerm 7 hrs monogerm 4 hrs
Radish	*Raphanus sativus*	TP ; BP	20-30;20	4	10	Prechill
Turnip	*Brassica rapa*	TP	22-30 ; 20	5	7	Prechill, KNO_3
LEGUME VEGETABLES						
Broad bean	*Vicia faba*	BP; S	20	4	14	Prechill
COLE CROPS						
Cabbage, Knol-kohl	*Brassica oleracea*	TP	20-30; 20	5	10	Prechill, KNO_3

Cauliflower , Broccoli	*B.oleracea var. botrytis and var. Italica*	TP	20-30;20	5	10	Prechill, KNO_3
Chinese cabbage	*B.pekinensis and chinenss*	TP	20-30;20	5	7	Prechill

TP-Top of the paper; BP - Between papers; 20-30 - Alternate temperature; 20; 25 - Constant temperature.

Note:-

1. Pre chilling: The replicates for germination are placed in contact with the moist substratum and kept at low temperature (between 5^0 and 10^0C) for upto seven days for all agricultural and vegetable seeds.
2. Potassium nitrate (KNO_3): Instead of water 0.2 % KNO_3 solution (prepared by dissolving 2 g KNO_3 in one litre of water) is used to saturate the germination substratum at the beginning of the test. Water is used for moistening thereafter.
3. Gibberellic acid (GA_3): Required concentration should be prepared. For preparing 1000 ppm solution dissolve 1 gm GA_3 in 1000 ml of H_2O; for 500 ppm dissolve 500 mg in 1000 ml of water; and for 100 ppm, 100 mg should be dissolved in 1000 ml of water. When concentration of GA_3 is not mentioned, any concentration ranging from 100 to 500 ppm should be used. Seeds should be soaked in required concentration of GA_3 for 17 hrs at room temperature, dried on the laboratory table and put for germination.

SEEDLING EVALUATION

The seeds placed for germination test are evaluated for germination after the germination period. Germination capacity of the seed lot is determined based on the evaluation of seedlings which is based on the presence of specific combination of the essential structures. The essential structures include root system (primary and seminal roots), shoot axis (hypocotyl, epicotyl and mesocotyl) and cotyledons.

Classification of seedlings

Based on the development of essential structures, seedlings are classified into:

- Normal seedlings (intact seedlings, seedlings with slight defects, with secondary infection);
- Abnormal seedlings (damaged, deformed, deranged, decayed and diseased seedlings)
- Fresh un germinated
- Hard seeds and
- Dead seeds

The fresh un-germinated or hard seeds and abnormal seedlings should be evaluated at the end of the test period. The stage of the development of the essential structures must be sufficient to permit detection of any abnormal seedlings. It may also be necessary to remove the seed coat and separate the cotyledons in order to examine the plumule in species where essential structures are still enclosed at the end of the test.

a) Normal seedlings: It is necessary to separate the normal seedlings, which are counted in the percentage germination, from any abnormal seedlings. To achieve uniformity in evaluating normal seedlings, they must conform to one of the following definitions:

a. Seedlings which show the capacity for continued development into normal plants when grown in good quality soil and under favourable conditions of water supply, temperature and light.

b. Seedlings which possess all the following essential structures when tested on artificial substrata.

The following categories of seedlings are regarded as normal seedlings:

b) Intact seedlings: A well developed root system consisting of a long primary root ending up with fine tip and presence of seminal roots (atleast two) instead of one primary root in Poaceae.

- In Poaceae family, a well developed primary leaf within or emerging through coleoptiles or an intact epicotyl with a normal plumular bud.
- In dicots, a well developed shoot axis consisting of straight, slender and elongated hypocotyls and intact epicotyl (without damage to the conducting tissue).
- One cotyledon for seedlings of monocotyledons and two cotyledons for seedlings of dicotylcdons.

c) Seedlings with slight defects: A primary root with slight defects provided the damage or the defect does not affect the conducting tissues.

- Seedlings of *Pisum, Vicia, Phaselolus, Lupinus, Vigna, Glycine. Arachis. Gossypium. Zea* and all species of Cucurbitaceae, with slight defect in the primary root and with well developed secondary roots and lateral roots to support the seedlings in the soil can be considered as normal seedling.
- Seedlings with superficial damage or decay to the hypocotyls, epicotyl or cotyledons which is limited in area and does not affect the conducting tissues.
- In dicots, seedlings with one cotyledon can be regarded as normal.
- Seedlings with primary leaves with limited damage are regarded as normal seedlings.
- Coleoptile with slight twist can be considered as normal seedlings.
- Decayed or damaged seedling, provided, the infection should not be from parent seed (only from the secondary infection) and the essential structures are well developed.
- Seedlings of tree species having epigeal germination when the radicle is four times the length of the seed. Provided all structures which have developed appear normal.

II. Abnormal seedlings

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

a) General

Seedlings with the following defects shall be classed as abnormal:

i. Damaged seedlings; seedlings with no cotyledons; seedlings with constrictions, splits,

cracks or lesions which affect the conducting tissues of the epicotyls, hypocotyl or root; seedlings without a primary root of those species where a primary root is an essential structure, except for *Pisum, Vicia, phaseolus, Lupinus, Vigna, Glycine, Arachis, Gossypium, Zea* and all species of Cucurbitaceae, when several vigorous secondary roots have developed to support the seedling in soil.

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

iii. Decayed seedlings: Seedlings with any of the essential structures so diseased or decayed that normal development is prevented, except when there is clear evidence to show that the cause of infection is not the seed itself.

iv. Seedlings showing cotyledon development from the micropyle, or radicle development from a part of the seed other than the micropyle.

b) Special categories of abnormal seedlings

The three main categories of abnormality, damage, deformity and decay, outlined in the previous section, can be further classified into categories as follows:

i. Roots

- No roots, in *Avena, Hordeum, Secale* and *Triticum* or one seminal root only.
- Primary root (or seminal roots in Gramineae) short and stunted.
- Primary root thin and weak, too short or too long.
- Primary root short and stunted, or short and weak, or spindly; secondary roots weak.
- No primary root or no well-developed secondary roots.
- Seminal roots short and weak, or spindly, or watery.
- Primary root split longitudinally, or damaged with secondary roots weak.
- Radicle with no root hairs.
- Radicle or primary root brown in colour.

ii. Hypocotyl and Epicotyl

- Hypocotyl short and thick, or twisted, or curled over, or watery.
- Epicotyl or stem with constriction, grainy lesion, or open split likely to interfere with the conducting tissue.
- Hypocotyl with constriction, grainy lesion, or open split likely to interfere with the conducting tissues.
- Epicotyl or stem short and thick or twisted round the main axis, or curled over along the main axis.
- No terminal bud.
- Two shoots which are short and weak, or spindly.
- No primary leaves, with or without terminal or axillary buds, or with more than half the total area of the primary leaves missing or not capable of functioning normally, or with one primary leaf and evidence of damage to the shoot apex.

- **Goose neck seedlings:** Seedlings with bent hypocotyl which affects the functions of leaf and shoot.

iii. Coleoptile (Gramineae)

- No green leaves.
- Short leaves extending less than half the length of coleoptiles.
- Leaves shattered or split longitudinally and/or coleoptile with a split easily visible to the naked eye, or abnormal coleoptile development due to damage.
- Plumule spindly, or pale, or watery.
- Plumule short and thick, usually with short or stunted seminal roots.

iv. Cotyledons (Dicotyledonous species)

- None
- One, with evidence of damage to the shoot apex.
- Poorly developed leaf-like cotyledon in Allium, without a definite bend, or "knee".
- Enlarged, with short hypocotyl.
- Physiological necrosis - as in (iv)h.
- Grey in colour
- Swollen and blackened
- More than half the total area broken off, or covered with spots or darkened areas, or with open splits if development as a whole is out of proportion compared with that of a normal seedlings germinated at the same time.
- **Bald head:** Produced in cotton and groundnut seedlings where the seed coat is still attached to the cotyledons preventing the opening of cotyledons which affects the development of seedling.

v. Decay

- Decayed cotyledons.
- Decayed hypocotyls.
- Decayed epicotyls or stem
- Decayed plumule, or decay at point of attachment between seedlings and endosperm, or discolouration of the coleoptiles which has penetrated to the leaves.
- Decayed primary root (except secondary infection by *Phoma betae)* or seminal roots in the Gramineae.
- Decay or discolouration at point of attachment between cotyledons and seedling axis, or adjacent to the shoot apex.
- Completely decayed seedling.
- **Other abnormalities**
- Seedlings short and weak, or spindly, or watery.
- Frost damaged seedlings with grainy Coleoptile or a plumule which is weak and spirally twisted.
- Entirely white seedling in the Graminease and Liliaceae
- Completely shattered seedling.

III. Hard Seeds

Seeds of Leguminosae, *Gossypium,* and *Hibiscus,* which remain hard at the end of the prescribed test period because they have not absorbed water due to an impermeable seedcoat, are classified as hard seeds. The percentage of hard seeds shall be reported separately from the percentage germination on the analysis Certificate.

IV. Fresh Ungerminated Seeds

Seeds, other than hard seeds, which imbibe water but do not germinate (due to defects or physiological disorders etc.) for want of some external treatments or conditions (i.e. dormant seeds) are classified as fresh ungerminated seeds and must be reported separately from the percentage germination. They become viable after the appropriate treatment for dormancy This occurs mostly in freshly harvested seed lots. They must be reported separately from the percentage germination.

Seeds which have just started to germinate at the end of the test period should be referred to the Section Leader.

V. Dead seeds

Seeds which at the end of the test periods are neither hard, nor fresh and have not produced any part of the seedlings are considered dead. If pressed, inner content oozes out due to decaying.

VI. Others :

Unfertilized, embryo less seeds, empty seeds etc.

VII. Multiple Seed Structures

Multiple seed structures of ***Beta vulgaris*** **and** ***Tetragonia expansa,*** schizocarps of *umbelliferae*, and multiple florets of *Chloris gayana, Arrhanatherum elatius, Dactylis glomerata,* and species of *poa* shall be tested as single seeds. The result of the test indicates the percentage of structures which have produced at least one normal seedling. The average number of seedlings produced by 100 seed structures may also be reported at the discretion of the testing station.

A tree seed giving rise to multiple seedlings as a result of polyembryony shall be counted as a single seed in the germination test. When the percentage of tree seeds with multiple embryos exceeds 5, the actual percentage should be shown on the Analysis Certificate.

Calculation and expression of result

Results are expressed as percentage by number.

$$\text{Germination (\%)} = \frac{\text{Number seeds germinated} \times 100}{\text{Number seeds on tray}}$$

When four l00-seed replicates of a test are within the maximum tolerated range, the average represents the percentage germination to be reported on the Analysis Certificate.

The average percentage is calculated to the nearest whole number. The total % of all the category of seeds (normal, abnormal. dead hard, fresh ungerminated) should be 100.

Reporting of result

The following items shall be entered in the appropriate space of the analysis certificate when reporting the result of a germination test:

1. Kind of variety
2. Date of testing
3. Duration of test
4. Percentage of normal seedlings, abnormal seedlings, hard seeds, fresh seeds and dead seeds. If the result for any of these categories is found to be nil, it shall be entered as 0

The following additional information shall also be reported:

(a) In all cases

1. Substrate and temperature used.
2. Any special treatment or method used for promoting germination.
3. The germination percentage obtained within the prescribed time, if the germination period has been extended beyond the period indicated.
4. The second result obtained when duplicate tests are indicated in Table 5A.

(b) Upon request

1. The result of any additional test,
2. The viability of ungerminated seeds and method used to determine it.
3. Categories of ungerminated seeds and methods used to determine them.
4. With multi-germ seed units: number of normal seedling produced by 100 units; proportion of units producing one, two or more than two normal seedlings.

Unsatisfactory results:

The result of a germination test is considered unsatisfactory, and is not to be reported under the following circumstances:

1. When the range in results for the 100 seed replicates exceeds the maximum tolerated range given in the tolerance table.
2. When there is an evidence that the results may not be reliable because of wrong test conditions, errors in seedlings evaluation or inaccuracies in counting or recording the results.
3. When there is evidence that the result may not be reliable because of dormancy, phytotoxicity, or the spread of fungi or bacteria.

Retesting

The result of a test shall be considered unsatisfactory and shall not be reported and a second test shall be made by the same or an alternative method, under the following circumstances:

1. When dormancy is suspected (fresh un-germinated seeds).
2. When the result may not be reliable because of phyto-toxicity or spread of fungi or bacteria
3. When there is difficulty in deciding the correct evaluation of a number of seedlings.
4. When there is evidence of errors in test conditions, seedling evaluation or counting.
5. When the range for the 100-seed replicates exceeds the maximum tolerated range

Reasons of variation in the germination test results

1. Poor sampling *i.e.* non uniform representative sample, random sampling error
2. Poor equipment, including variation in temperature, light and humidity in germinator
3. Substrata quality: Toxicity or impurities in Paper or sand
4. Use of stored or old germination papers
5. Incidence of fungi or bacteria or others in the seed
6. Improper phytosanitary conditions of laboratory, containers and germinators
7. Effect of seed treatment
8. Untrained or inexperienced analysts
9. Inaccurate counting of seed or seedling
10. Observation before or after prescribed time
11. Interpretation of seedling performance: Normal/abnormal and dead and fresh un-germinated.

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Determination of Seed Viability

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Seed Viability

"Seed viability can be defined as the ability of the embryo to live, grow and develop into a seedling under favorable environmental conditions".

Or

Seed viability refers to state of aliveness

Objectives of seed viability

- To obtain quick estimation of viability of seed samples or of individual seeds remain ungerminated at the end of germination test.
- To determine the rapidly viability of the seeds of certain species which germinate very slowly or show high degree of dormancy.

Factors affecting seed viability

1. Internal factors

- ✓ Immature and small seeds within a seeds ,within a seed lot do not store as well as mature and large seeds within a seed lot (Wien *et al*)
- ✓ Several kinds of environmental stresses during seed development, and prior to physiological maturity, can reduce the longevity of seeds.
- ✓ The physical condition and physiological state of seeds greatly influence their life span.
- ✓ Seeds that have been broken, cracked, or even bruised deteriorate more rapidly than undamaged seeds(McDonald 1985;Priestley 1986)

2. Genetic factor

Seeds of some species are genetically and chemically equipped for longer storability than others under similar conditions.

Most long-lived seeds belong to species possessing hard, impermeable seed coats. Seeds of canna (Sivoriet *et al.*, 1968), Lotus (Wester 1973), and Lupinus (Porsild and Harrington 1967) have been reported to be viable even after 500 years.

Seeds of other species are characteristically short lived; these include vegetables such as lettuce, onion, and parsnip and also agronomic crops such as Rye. Generally seed species possessing high oil content do not store as well as those with low oil content. For ex, whole wheat seeds contain only about 3% oil, but their embryo portion has about 27% oil. Seeds of different species may also be chemically similar but have different storability due to differences in genetic potential. For example, Chewings Fescue and annual rye grass seeds are similar in appearance and chemical composition; however rye grass seeds have much better storability under comparable conditions. Genetic differences in storage potential are not limited to seeds of different species, It also occur among cultivars. The

bean cultivar black Valentine stores better than Brittle wax (Toole and Toole 1953). However the environment strongly alters the genetic potential for seed longevity.

Relative humidity and temperature

Temperature

At a temperature of 0^0 c, formation of intracellular ice crystals can disrupt membrane integrity & contribute to seed deterioration. However Seeds with moisture levels below 14% do not form ice crystals. It should be noted, however, that at 14% initial moisture, seeds stored in cold rooms below 0^0c will likely gain moisture. Most cold rooms have a high relative humidity & seeds achieve equilibrium with relative humidity after a brief period of storage. Thus seeds stored at low temperature must be in conditions in which the relative humidity is controlled or placed in moisture –proof containers to avoid increase in moisture content & increased deterioration.

Seed Moisture

Seeds contain moisture above 14% begin to exhibit increased respiration, heating, and fungal invasion that destroy seed viability more rapidly. Below 5% seed moisture, a breakdown of membrane structure hastens seed deterioration. This probably a consequence of reorientation of hydrophilic cell membranes due to loss of the water molecules, necessary to retain their configuration. Thus, studies standardized that storage of seeds Cereal (10-12 %), Pulses (7-8 %), Vegetables (4-5 %), Oilseeds (7-8 %) appears to be ideal; for maximum longevity.

Viability tests

- Standard Germination test
- Tetrazolium test
- Excised embryo test
- Fast green test
- Conductivity test

1. Standard germination test

The emergence and development of seedling to a stage where the aspects of its essential structures indicate whether or not it is able to develop further in to a satisfactory plant under favorable conditions in soils (ISTA, 1985).

Steps of germination test

- Putting of seeds
- Keeping in germinators at optimum condition
- Period of test -Days to count -Ist and II nd count
- Seedling evaluation
- Calculation of results
- Reporting of results

Seedling evaluation:

- CONCEPT: Evaluation should be done only after all essential structures are fully expressed & evaluate as NS, AS, HS, FUG & dead seeds
- Normal seedlings (NS) : Seedlings showing continued capacity for development into normal plant when grown in good quality soil under favourable conditions
- NS Categories (ISTA)
- Intact seedlings :Seedlings with essential structures well developed in all proportions, healthy, showing balanced growth
- Slight defective Seedlings : Seedlings with slight defects in their essentials structures provided they show normal vigorous, balanced growth in comparison with intact seedlings
- Seedlings with secondary infection: Seedlings with clear evidence of secondary infection are classified as NS provided all essential structure are otherwise normal.
- Seedlings with secondary infections even if seriously decayed or diseased are considered as normal

2. Tetrazolium test

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

Viability: Seed viability indicates that a seed contains structures and substances enzyme system which give it the capacity to germinate under favorable condition in the absence of dormancy.

Objectives:

1. To obtain quick estimation of viability of seed samples or of individual seeds remained ungerminated at the end of germination test.
2. To determine the rapidly viability of the seeds of certain species which germinate very slowly or show high degree of dormancy.

Equipments and chemicals required:

a) One percent solution (W/V) of 2, 3, 5 Triphenyl tetrazolium chloride (TZ) or bromide.
b) Potassium dihydrogen phosphate.
c) Disodium hydrogen phosphate.

Conditioning Media: Blotter, paper towel or beaker.
Cutting or piercing devices: Razor blade, dissecting knives and needles.
Staining dishes: Watch glasses/petridishes.
Magnifying devices: Hand lens and stereoscopic microscope.

Preparation of buffer solution

Solution 1 – dissolve 9.078 g KH_2PO_4 in 1000 ml water
Solution 2 – dissolve 11.876 g Na_2HPO_4 in 1000 ml water
Mix 400 ml of solution 1 with 600 ml of solution 2 to get a liter buffer solution of neutral pH.

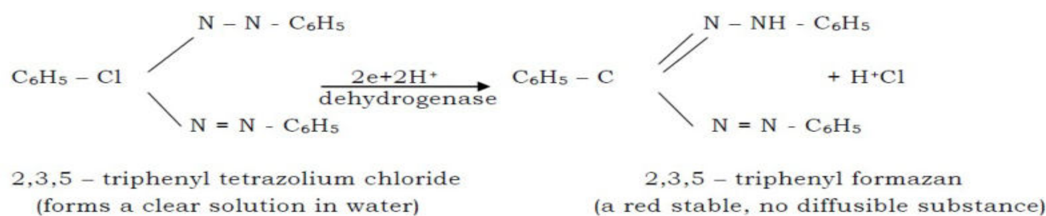
To get 1% of TZ solution, dissolve 1 g of TZ salt in 100 ml of buffer solution. (The one percent solution is used for seeds that are not bisected through the embryo, while the 0.1 percent solution is used for seeds in which the embryo is bisected. Other low concentration such as 0.2 percent and 0.5 percent are some time used instead of 0.1 percent solution).

Straining: The prepared seed should be placed in suitable container (small beaker, Petri-dishes, watch glass, etc.) and place these container in a dark ward place. The staining time varies for different kinds of seed, different methods of preparation and different temperature (less than one hour to approximately eight hours).

A sample is satisfactorily stained when tissue develops interpretable staining characteristics and the analyst can sense 'embryo conditions. When observations indicate that a sample has stained sufficiently, the TZ solution should be discarded and observation can be made.

Principle: when the seeds are soaked in colorless solution of 2, 3, 5 triphenyl tetrazolium chloride (TZ) or bromide. it interferes with the reduction process of living cells within the seed tissue and accepts hydrogen ions from the dehydrogenase enzymes. Due to hydrogenation, (H+ ions transfer) triphenyl tetrazolium chloride get reduced into a red coloured compound, non diffusible substance called formazan. In the living cells. Since, the reactions takes place within the respiring (living) cells and the formazan is no diffusible a clear topography of living and nonliving areas within the seed can be developed by using proper procedure. For this reason, the test is designated as the topographical tetrazolium test.

The reaction as follows:



Evaluation of sample: The sample is ready for evaluation when it is stained. Observe the staining pattern and calculate the percentage of viable seed.

1. On the basis of staining of embryo

a. Embryo completely stained- viable.
b. Embryo unstained-non viable.
c. Plumule or radical unstained-non viable.

2. Assessment on the basis of cotyledon

a. Complete staining-viable.
b. Unstained-non viable.
c. Necrosis –evolution on the basis of category.

3. Assessment on the basis of necrosis

a. Unstained tissue at the attachment of the embryo-non viable.

b. Unstained tissues are away and are not connected with embryo-viable.

4. Assessment on the basis of color intensity

a. Dark red – vigours seed.
b. Pink color -weak seed.
c. Dark red fractured- non viable.

5. Specific evaluation

A. Germinable seeds of cereals

a. Well developed embryo with an fractured normal cherry red stain.
b. Necrosis with the upper or lower ends of the scutellum.
c. Radical unstained but embryonic axis stained.

B. Non germinable seeds f cereals

a. Whole embryo unstained.
b. Scutellum node unstained.
c. Major area of coleoptiles unstained.

C. Germinable seeds of legumes/oil seeds

a. Non fractured red colored embryo and cotyledon.
b. Normal red colored embryo with only one normal cotyledon.
c. Normal red colored embryo with half or more than half of both the cotyledons attached to embryo are of red color.

D. Non germinable seeds of legumes

a. Embryo completely unstained.
b. Fracture at radical or plumule with dark red line.
c. Plumule or radical tip unstained.
d. More than ½ part of both the cotyledons attached to embryo are colorless.
e. Attachment of embryo to cotyledon is unstained.

Calculation: the results are reported as percentage of viable seeds in relation to total seed tested.

Advantages of TZ:

1. Quick estimate of viability can be obtained (within 12-20 hrs.)
2. When the seed is dormant or very slow in germination, a viability test is extremely useful.
3. Seeds are not damaged (in dicot only) in analysis, therefore they could be germinated.

Disadvantages of TZ:

1. It is difficult to distinguish between normal and abnormal seedlings.
2. It does not differentiate between dormant and non dormant seeds.

3. Excised embryo test

- The excised embryo test is similar to germination tests in that it measures the quality of the seed by their actual germination.
- In addition it allows some measure of the embryo dormancy to be made, by counting those seeds which, although not growing normally, have grown slightly, remained firm and have kept their colour for the test period.
- The test is not valid for previously germinated seeds and must not be applied to samples which contain any dry germinated seeds.
- The success of the test requires considerable skill and experience in the operator and the ISTA rules restrict it to only a few species

4. Fast green test

- The fast green test reveals physical fractures in the seeds such as corn.
- Seeds are soaked in a 0.1% fast green solution for only 15-30 seconds.
- During this period, the fast green penetrates any area of the seed coat which has been fractured and stains the endosperm green .
- After the soak period, the seeds are washed and the fractures then become apparent (visible) in the seed coat.

5. Conductivity test

- The conductivity test is a biochemical test, which measures the amount of electrolytes, which leach through the seed coat or fruit coat of the intact seed.
- A higher conductivity may indicate a low viable seed lot.
- The expected readings for a conductivity test will vary greatly from crop to crop.
- It is most useful for peas, soybean samples, and a lesser degree for corn.

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Post Harvest Handling of Seeds

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Seed is one of the most important inputs for sustainable agriculture. Quality of seed affects both yield and credibility in the market. Unlike in grain, extreme care and vigilance is required in seed to avoid mechanical mixing of crop varieties during post harvest stages such as threshing, winnowing, drying, Pre-cleaning, grading, packaging, storage and marketing. Many a time carelessness as well as ignorance at any stage cause colossal loss in seed quality and market value. Hence in-depth knowledge of post harvest care and improvement in physical purity of seed is most important.

The objective of seed processing is to achieve clean, pure seeds of high physiological quality (germinability) which can be stored and easily handled during succeeding processes, such as pretreatment, transport and sowing. Processing includes a number of handling procedures, where applicability differs e.g. according to seed type, condition of the seeds after harvest and potential storage period. Seed cleaning typically consists of a series of processes during which impurities are gradually removed and the seed lot concurrently achieves a progressively higher purity (Fig-1). The type, order, and adjustment of the processes depend on seed type and type of impurities. During seed processing, contaminants are removed to a level that meets the industry wide minimum seed certification standards, failing which, they may be discarded or blended with a relatively better lot of the same variety. Contaminants are removed by procedures utilizing machines which exploit the differences in physical characteristics of the desirable seed and other components in the mixture. These physical properties include but are not limited to length, width, thickness, shape, density, terminal velocity, drag coefficient, reflectivity, surface texture, electrical conductivity and resilience. Seed separators are designed to utilize the difference in a single physical property or a combination of physical properties of the seed.

Concept of Separation Processes:

Separation and purification of materials forms an important process in post harvest handling of agricultural products. Naturally occurring processes are inherently mixing processes and have led to the reverse procedure of separation processes which are becoming the most challenging categories of engineering problems. Mechanical separations are applicable to heterogeneous mixtures. Broadly, a separation processes a mixture of substances in two or more products which differ from one another in composition. The separation is caused by the addition of a separating agent which may be in the form of energy. Need for separation accounts for the most of the production cost of a pure substance. Often separation itself can be the key function of the entire process e.g. grain cleaning. To a large extent man's ability to ease food shortage depends upon his technical knowledge and capacity to extract and separate essential food materials from the new or inexpensive sources. From the above considerations, it is apparent that much

careful thought and effort must go into understanding and improvement of various separation processes.

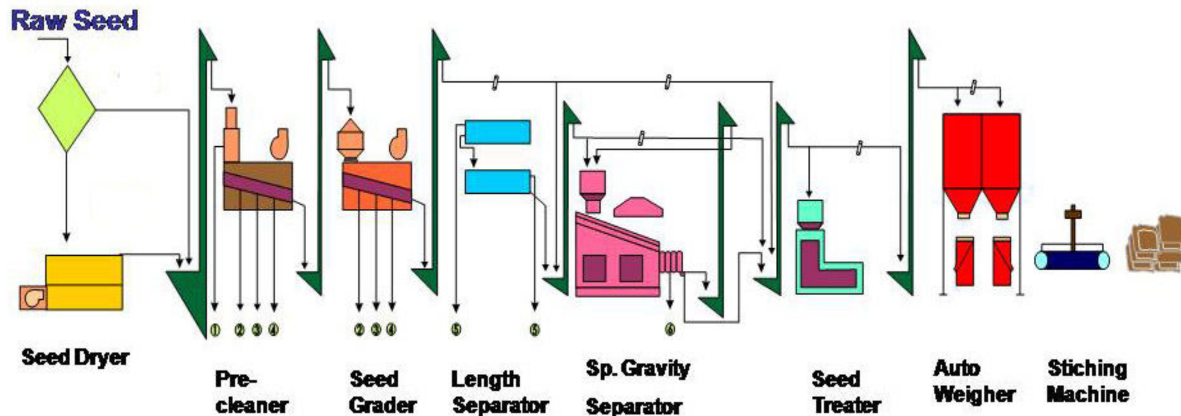

Fig-1: Flow diagram of modern seed processing

Methods of Seed Separation:

Improvement in seed separation technology from simple hand picking and domestic hand screen to present day methods runs parallel to the story of civilization. A modern seed processing involves moving the field produce through a series of machines which perform specific operations and pass on the product to the next machine after discharging the reject. A well designed seed processing plant is laid out to permit by passing any machine without interrupting the product flow. Many types of seed cleaning machines are used to remove contaminants from the harvested-threshed seed.

Air-Screen Cleaner:

The air-screen cleaner is the most widely used machine. It is an essential unit operation in seed processing plant. The simplest mechanical method of separating particulate solids, the class to which most agricultural seeds and food grains belong, is by passing than over screens which are stationary or reciprocating and are set at a slight downward slope, so that small particles will pass through and larger materials will tail over them. In combination with air-fans or blowers, the screen machine provides adequate conditioning for some seed crops. Such machines work by taking advantage of dimensional and aerodynamic differences. Agricultural screens are constructed of perforated metal or woven wire mesh. Hole shapes in perforated screens are usually round, triangular, oblong or rectangular, Openings in wire screens are square or rectangular, their size being represented by mesh numbers. Round hole screens are identified by a number denoting diameter of the perforation. In India, these numbers indicate the diameter in millimeters. Rectangular or oblong holes in perforated screens are identified by two numbers describing the width and length of the slot. Selection of the screen depends on the seeds

being handled. Screen opening sizes used for different crops have been prepared and are available in literature. Screens with various sizes and shapes of holes drop some particles and retain others depending mainly on the width and thickness of particles and, to a lesser extent on their length. Pneumatic separators or air columns exploiting aerodynamic differences are used to remove dust, chaff or other light contaminants. The air system in air-screen machine operates in this manner. As a finishing machine it can remove light, immature, shriveled or damaged seeds from already cleaned good seed lots. Air screen combinations are extensively used in grain combining and threshing.

The air screen machine in general employs three cleaning elements: aspiration, scalping and grading. The light seeds and chaffy materials are removed from the seed through aspiration. In scalping operation, the good seeds are dropped through top screen opening and the larger materials (trash, clods etc.) are carried over the screen into the rejection spout. In grading operation, the good seed ride over screen openings, while smaller particles (under size, cut shriveled, broken seeds) drop through.

Feed hoppers of air screen cleaner cum grader are of three types: Roll feed hopper consists of a container to receive the seed, hopper flights and auger to spread the seed across the width of the hopper and a revolving fluted roll in the bottom of the hopper that feeds and even steady flow of seed to the top screen and distributes the seed across the full screen width. In roll feed brush hopper a rotating shaft pulls trash of seeds down to the revolving fluted roll and a tough fibre brush to prevent clogging. In the metering hopper a shaft width specially bent rod is used to spread the seed. Other special purpose variants are designed to handle special seeds.

Principles of operation:

In a typical two screen seed cleaner cum grader, as the seed is delivered by the feed hopper the air blast removes light weight seed and chaff, scalping screen remove material larger than the crop seed; grading screen dropout material smaller than the crop seed. In a four screen machine, the 4 screen do the following operations: (a) 1st screen- scalping, (b) 2nd screen- grading, (c) 3rd screen- close scalping, (d) 4th fine grading. At the seed drop off the gravity screens they fall through the lower air separation to remove residual light seed and trash.

Length Separator:

Length separators are designed to lift and remove the short fraction from a varied length mixture by exploiting the difference in the largest dimension of the product and the reject. These are two types of length separators, the indented disc separators and indented cylinder separators. Both lift out short particles out of a seed mixture with a given pocket or indentation and a relatively cleaned product is pushed further. The indented disc separator consists of a series of indented discs, mounted together on a rotating horizontal shaft. Each disc is designed with an open centre and numerous undercut recesses on each face. The broken seeds and the material shorter than the crop seed are lifted by the indents and are delivered into a trough at the side of the machine. Discs of increasing pocket sizes are normally provided on the shaft so that the particles of increasing lengths are removed selectively. The long seed that does not match the pockets is pushed by the incoming seed through the open centre of the disc and is discharged at the outlet.

The indented cylinder separator consists of a rotating cylinder and an adjustable trough. The inner surface of the cylinder has closely spaced indents. The seed mass to be handled is fed at one end and lies at the bottom of the cylinder. As the cylinder rotates on its axis the short seeds are lifted from the mixture by indents. Thus at some point before reaching the top of the rotation, the seeds fall out from the indents, because of the tilting of the later. Actually, the seeds resting in the indents lose balance and are eventually received in the adjustable trough from where they are conveyed out by an auger. The long seed which is not lifted by the indents gradually move through the cylinder end are discharged to a separate spout at the other end of the cylinder. The quality of separation depends on the position of the trough and the speed of the cylinder.

Specific Gravity Separator:

A specific gravity separator consists of two key components - air chest and the deck. Air chest houses fans and motor. The deck is mounted above the chest. The deck is a rectangular or triangular table covered with a porous cloth or wire mesh and inclined in two directions. The gravity separator classifies components of a mixture mainly according to density. Separation is caused in two steps. Seed mixture introduced at the back of the porous deck is stratified by the low pressure air coming through the deck. Low density particles tend to float and form a layer at the top and the high density particles sink to the bottom layer. Fractions of intermediate density, assume intermediate position. For proper identification of different density fractions, the seed lot must be well screened before hand so that all particles are of the same size. The seed should be dust free. An aspiration canopy is installed above the feed corner to further suck up any residual dust. The oscillating motion of the deck moves the high density particles laterally towards the uphill side at the deck. Simultaneously the floating low density material moves downhill by gravity. As the seed mixture layers travel from the feeding corner to the discharge end of the deck, a continuous gradation of particles takes place ranging from the low density ones at the lower side of the deck to the high density ones at the upper side. Adjustable splitters divide the output into number of density fractions needed. For deck covering a closely woven material for small seeds and a coarse weave for large seeds is used. Typical covering materials are small hole perforated metal and wire mesh. The coverings are supported by a deck frame, which serves as the top of the air chamber and helps to equalize the flow of air through the seed mass. Feed rate, air flow rate, deck angles and frequency of stroke are major adjustments. These adjustments are interrelated.

Seed Refining:

To further refine the seed, machines have been developed to take advantage from additional differences in physical properties. The electrostatic separator exploits the difference in the electrical characteristics of the seeds and contaminants. The quality of separation depends on the relative availability of the components in the seed mixture to conduct electricity or to hold electrical charge on surface. A spiral separator senses the ability of components to roll. This is very simple machine and operates completely by gravity. It has no moving parts and needs no prime mover. The endless draper belt separator utilizes surface texture differences to separate rough seeds from the smooth ones. A magnetic separator requires certain pre treatment of the feed mixture. Iron power or a

magnetic fluid is added. Variation in seed coat characteristics is utilized. The iron is selectively adsorbed by rough, broken, cracked porous or sticky components making them more reactive than the smooth components. A colour separator acts on differences in reflective properties. The components of the mixture must be cingulated for individual sensing by the photoelectric cells. To scale up the throughput multi-channel machines are required.

New Emerging Technologies:

Modernization of agriculture causes demand for higher quality seeds and invites application of new technologies to seed conditioning. This needs removal of all contaminants even when the physical property difference is very slight. This emphasis has led to the investigation of measurement system for physical properties and development of systems for improved seed conditioning. With the advent of microprocessors and the rapidly expanding application of technology, seed conditioning is beginning to benefit as the use of computers is integrated into the new equipments. Machine vision system (MVS) is being used for seed conditioning. The feasibility of the application was shown for identifying seeds of different colour, size and shape. The MVS can also be used to detect stress cracks in certain seeds. There appears a need to develop expert systems for modern seed processing and once a system is made available, the performance and the status of an average worker can be raised to the level of an expert.

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Detection and Identification of Insect Infestation in Seed Lots

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Seed health testing is the pre-requisite for quality assurance in seed production, supply and finally its success as healthy crop. Since seed produced in one season is used in the next season (s), it passes through various conditions prevailing during storage period ranging from 6-7 months to more than a year in case of carry over stock and several years for buffer stocks. During storage abiotic (temperature, humidity, oxygen) and biotic (birds, rodents, insects, microbes, type of seed and its moisture content) factors influence the health of seeds. Among the biotic factors, insects play vital role in determining the quality of stored seed.

Further, it was found that insects damaging embryo cause maximum loss to seed viability and vigour both compared to damage to the endosperm and un-damaged seed.

Seed Portion Damaged*	Germination (%)			Vigour index
	Normal	Abnormal	Dead	
Embryo (B)	4.1	7.8	88.1	85.3
Endosperm (A)	57.3	6	36.7	816.9
Undamaged	96.6	2.2	1.2	1384

***See picture (A & B) for Damages**

Important insect-pests of stored seeds:

The majority of insect pests belong to the orders Coleoptera (beetles & weevils) and Lepidoptera (moths). These insects can be categorized as major or minor pest according to the damage to seeds/grains caused by them and also on the basis of their feeding behaviour.

(a) ***Primary feeders***: These insects mostly lay eggs inside or on the seed and spent a part or entire larval and pupal life inside the seed. They contribute significant loss of germination which is not detectable outside e.g. rice weevil, lesser grain borer, pulse beetle, khapra beetles, Angoumois moth etc.

(b)***Secondary feeders***: This group of insects feed on germ and endosperm from outside. They may attack whole seed and damage the embryo portion or feed on the seeds if seed moisture is high, which has already been damaged /infested by other insects. These insects and their different stages are generally visible among the seeds e.g., red rust flour beetle, saw toothed beetle, Indian meal moth, etc.

The most commonly stored insect pests infesting seed lots and their host are mentioned below :

INTERNAL FEEDERS

Common name	Systematic position	Hosts
Rice weevil,	Sitophilus oryzae (Curculionidae, coleoptrea)	Rice, wheat, sorghum, barley, maize
Lesser grain borer	Rhizopertha dominica (Bostrychidae, coleoptera)	Rice, wheat and maize
Pluses beetle	Callosbruchus chinensis, C. maculates, (Bruchidae, Coleoptera)	Pulses, bean and gram.
Cigarette beetle	Lasioderma serricorne, (Anobiidae, Coleoptera)	Tobacco, wheat, peanut , cocoa, bean, cotton seed
Drug store beetle	Stegobium paniceum, (Anobiidae, Coleoptera)	Turmeric , coriander, ginger, dry vegetables and animal matters
Groundnut bruchid	Caryedon serratus (Bruchidae, Coleoptera)	Ground nut and other legumes
Angoumois grain moth	Sitotroga cerealella, (Gelechiidae, Lepidoptera)	Paddy, maize and wheat.
Potato tuber moth	Phthorimaea operculella (Gelechiidae, lepidoptera)	Potato, tomato, tobacco, eggplant and Beta vulgaris
Sweet Potato weevil	Cylas formicarius, (Apionidae, Coleoptera)	Sweet potato few other species of Ipomoea

EXTERNAL FEEDERS

Common name	Systematic position	Hosts
Khapra beetle	Trogoderma granarium, (Dermestidae, Coleoptera)	Cereals and groundnut pulses species and pulses cakes
Red flour beetle	Tribolium castaneum, T. confusum, (Tenebrionidae, Coleoptera)	Maize wheat, etc.
Saw toothed grain beetle	Oryzaephilus surinamensis, (Silvanidae, Coleoptera)	Dry fruits, rice , wheat, maize cereals and oilseeds
Rice moth	Corcyra cephalonica, (Galleridae, Lepidoptera)	Cereals, oilseeds nuts, dry fruits, rice and pulse
Tropical Warehouse Moth.	Ephestia cautella (Hb.) (Phycitiae, Lepidoptera)	Wheat , rice , maize sorghum, groundnut and species
Indian Meal Moth	Plodia interpunctella (Phycitiae, Lepidoptera)	Maize cereals dry fruits, groundnut, and cereals products
Grain mite	Acarus siro (L.) Order: Astigmata	Cereals grains, flour and other eatables

Sources of insect infestation under storage
The main sources of infestation are :

- **Field infestation :** Some of the insects like bruchids, *Sitophilus oryzae, Sitotroga cerealella* infest seed crops at reproductive stage in the field. They come along with the

harvested produce and multiply during pre-storage or storage period. The infestation is normally detected at the time of emergence of adults.

- **Godowns itself :** Insects or its stage(s) hiding in the cracks and crevices, electrical fittings etc. are the major source of infestation in the godowns.
- **Old gunny bags / receptacles/containers:** Insects or its stage(s) hide in the weavings or corners and infest the seed when stored in such bags/containers.
- **Vehicle:** Vehicles are also sources of infestation when in regular use. Now a day, containers are used for transporting large quantity of bagged seed for exports. Such containers need thorough cleaning and treatment to kill insects hiding in it. Infestation also may occur from machineries and threshing yards etc.
- Infestation may occur through wind current from nearby field
- Infestation from the birds nest
- Infestation from the ant and rodent burrows

Monitoring and detection of insect infestation

Detection of insect infestation is necessary because it ensures a supply of high quality and healthy seed to the farmers. It also serves as an early warning for taking appropriate control measures. Delay in detection may result in pest outbreaks, causing severe contamination of seed materials and quantitative loss. It also helps in assessment of effectiveness of fumigation and other pesticide treatments. Therefore, It is important to inspect regularly to detect the first signs of infestation or spoilage.

1. Visual Inspection : It includes inspection of the place for live flying or crawling insects during spring, summer or rainy seasons. Detection of live insects or its castings in sweeps and presence of flour deposits on bags caused by lesser grain borer. Presence of web in undisturbed places as a sign of lepidopteran infestation.

2. Light traps : Most insects are nocturnal and phototropic. Light traps detect presence and its build up. Light traps with an electrocution net kill insects that are attracted to it and help in controlling the adult insects. Mohan et al. (1994) in Tamil Nadu, used a 4W ultraviolet light (peak emission at 250 nm) set at 1.5 m above ground level in the alleyways and corners of godowns. This detected accurately the presence of *R. dominica*

3. Sticky traps: These help in early detection of insects, especially in the top of bins and helps in early prediction of infestation levels. Hagstrum *et al* (1994) observed that sticky traps correctly predicted the presence of lesser grain and rusty grain beetles in samples drawn from 79-86% of the bins.

4. Traps for crawling Insects: It provides a hiding place and are available in various designs. It can be used with pheromone lures for specific insects or food baits to enhance capture of multiple species.

5. Pheromone traps: Unlike light traps, these traps are baited with a synthetic chemical which influence an insect's behaviour. These chemicals are species specific and help in better monitoring of particular pests. Traps have also been found very effective in early detection of insects at low population levels. Therefore, this can help in detecting re-infestation Pheromone traps are now available with adhesive glue to which insects get stuck thus, helps in removing a proportion of the population (mass trapping).

List of synthetic pheromones of major storage insects for monitoring (M) or mass trapping (MT)

Storage insect	Scientific name	Main host	Pheromone component	Purpose
Khapra beetle	Trogoderma granarium	Stored wheat	Z, E-methyl 8-hexadecenal (92:8)	M/MT
Pulse beetle	Callosobruchus chinensis	Stored pulses	'Erection', a mixture of hydrocarbons, dicarboxylic acid	M/MT
Lesser grain borer	Rhyzopertha dominica	Stored cereal	Dominicalure 1: (S)-(+)-1-methyl butyl (E)-2, butyl (E)-2, 4-dimethyl -2-pentenoate	M/MT
Grain moth	Sitotroga cerealella	Stored cereal seeds	(Z.E.) 7,11-hexadecadienyl acetate	M/MT
Indian meal moth	Plodia interpuctella	Stored cereal seed	(Z.E.) 9,12 – tetra decadienyl acetate	M/MT

Detection of insect infestation in seed lots

1. Quantitative sampling: The aim of drawing random samples is to determine the mean value and the variability of the level of infestation or contamination in the seed lot. Ashman (1970) devised a tentative "sequel sampling" procedure, involving collecting a number of spear samples from several bags at random and then examined by sieving. It does not account for hidden infestation in the kernels. The sample number should not be less than the **square root of the total number of bags** until a 1 kg sample is obtained, and examined for insects by sieving.

2. Direct examination :Seeds are examined in the dry state with the help of a magnifying glass (10X) or stereoscopic microscope aided with light. Two replicates samples of 200 seeds each are visually examined. Live and dead adult weevils, beetles, moth larvae, grubs etc. are separated and counted. They are recorded as number of insects including all stages per weight of sample.

Insect-damaged seeds are separated and counted including those whose germ (embryo) has been scratched or eaten or have escape hole (s) or eggs adhered to them. Other seeds with no visible symptoms of insect injury are subjected to further tests to detect internal infestation.

Detection of internal infestation in seed

The number of internally infested seeds is added to the number of seeds found externally damaged by insect for final calculation. Special techniques to detect internal infestation are employed such as:

1. **Dissection method:** The seed is cut open or dissected or cracked with or without soaking in water to reveal internal infestation.

2. **Alkali or glycerin method:** Seeds are submerged in 10% solution of NaOH and boiled for 10 minutes or more depending upon type of seed. After decanting the NaOH solution, seeds are washed with water. The translucent seeds are then examined with a magnifying

glass. Those with visible internal infestation are cut open to confirm infestation. Alternatively, seeds can also be made translucent in lactophenol (dissolve 20 g phenol crystal in 20 ml luke warm distilled water, and then add 20 ml lactic acid and 10 ml glycerin) solution and follow the above mentioned steps.

3. **Flotation method** - This is also called as specific gravity method for detecting hidden infestation in whole grains. The density between sound (un-infested) and infested grains is exploited for the detection of infestation using salt solutions. Accordingly, when seed sample is immersed in a salt solution of appropriate density (normally 1.19 g/cm3), for about 10 minutes, the heavier un-infested seeds sink to the bottom while the lighter infested ones float. It can be used for detecting internal infestation in cereals and pulses seeds. The mixture consists of a solution of sodium silicate in water with specific gravity of 1.16, to which methyl chloroform is added after adjusting its specific gravity to 1.30 with deobase oil. When placed in a measuring cylinder, a distinct separation layer is formed between the two liquids, the lighter (sodium silicate) solution remain on the top. Seeds containing later stages of weevil larvae float on top of the sodium silicate solution, whereas seeds containing early stages of larvae or light weight seed float at the interface of the two liquids. Non-infested normal seed will sink to the bottom. In the presence of floaters, the degree of infestation can be estimated by the relation between number of floaters and size (number) of sample.

Flotation method for insect-hole bearing seeds- A sample of seed is placed in 2% ferric nitrate solution (dissolve 2 g hydrated ferric nitrate in 100 ml water and stir for 30 seconds). Seeds with an insect emergence hole will float while the rest will sink to the bottom. The number of floaters can be counted and infestation can be worked out. This technique is suitable for determining infestation in pulse seed.

Flotation separation by air- By progressively increasing the intensity of air stream by fan in vertical column, all insect-damaged kernels can be blown out in the first two fractions, from which no emergence had occurred. The detection of insect-damaged grain (i.e. those containing exit holes) can then be a relatively quick and efficient operation, and may speed up the exit-hole inspection procedure in commercial samples by a factor of ten or better.
It is a qualitative test and does not indicate the species or the specific life stage. The method is simple and quick. Low weight, shriveled seeds will also float with the infested seed during the test. Hence, it requires confirmation by dissecting the floating seed. Seeds with eggs or early larval stages cannot be detected because it will not float.

4. Staining Methods-

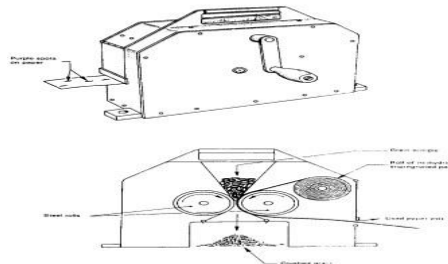
Staining, a chemical indicator technique is a direct method of establishing hidden living infestation in the seed. There are three types of Staining techniques,
The mucilaginous secretions of weevils (*Sitophilus* sp.) are stained with a suitable chemical compound. Weevils deposit their eggs inside the seed and plug the holes or egg cavities with saliva. Using suitable coloring agents, the egg plugs in grains can be stained and identified. The extent of infestation in a sample is estimated by the number of egg plugs observed. This technique is not applicable for other insects such as *R. dominica* and *S. cerealella* that oviposit outside the grain.

Detection of Insect Infestation by Staining Techniques

Chemicals used	Color spot	Reference
1. Specific for weevil egg plugs in grains		
Acid fuchsin	Cherry red	Frankenfeld(1948)
Gentian violet	Purple	Goossens(1949)
Berberine sulfate	Greenish yellow	Milner et al. (1950a)
2. Specific for entry holes in pulses		
Iodine-potassium iodide	Black	Frankenfeld(1948)
3. General infestation in whole grains		
Ninhydrin	Purple	Ashman et al. (1970) Dennis & Decker (1962)

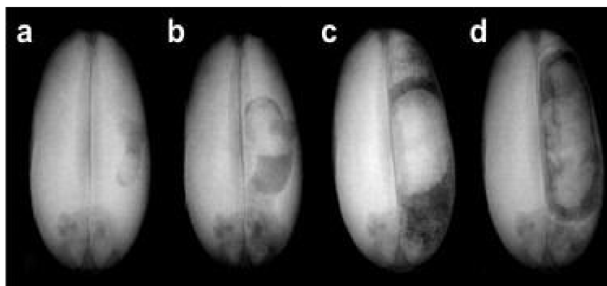
The ninhydrin method : The chemical indicator, ninhydrin (triketohydrindene hydrate) is used for detection of live insects inside the seed. When insect body fluid comes in contact with the ninhydrin-impregnated filter paper, it produces a "purple" colour.

In the United Kingdom, a small portable machine known as the ''Ashman-Simon infestation detector'' was developed. A counted number of seeds are fed into the machine. They pass into the folds of filter paper impregnated with 0.7% ninhydrin acetone solution, and are crushed when they pass through the rollers. The body fluid of insects within seeds oozes out that reacts with the chemical forming purple spots on the tape. Sometimes mild heating is required to develop clear spots. The machine operates at a speed of 45 cm or 300 kernels per minutes and it is claimed that it detects 5–10% of eggs and early larvae, 40–60% of middle age larvae, and 80–90% of mature larvae in cereals

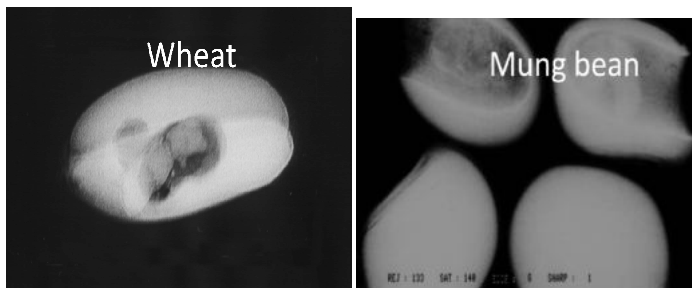


Apparatus for ninhydrin detection of hidden insect infestation "Ashman-Simon infestation detector"

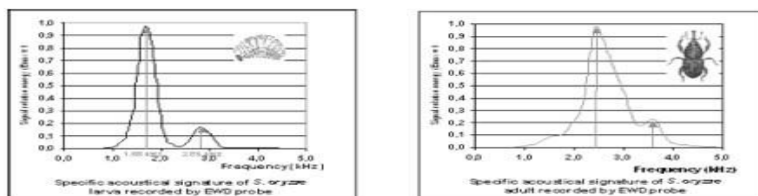
5. X-ray method: This is an accurate and rapid method of detecting internal insect infestation in a sample of seed and was described by (Milner et al., 1950). An X-ray machine operates at about 50 KW. Exposure period varies from seed to seed to get a good radiograph which reveals the presence of any insect inside the kernel. It is being extensively used by different countries, especially for quarantine purposes. X-ray manufacturers have developed a compact and suitable unit for this purpose such as X-ray Softex SB-40 manufactured by M/Nissei Commerce Ltd., Tokyo, Japan.



X-ray images of wheat seed infested by different stages of granary weevil (*Sitophilus granaries)*: small larva (a), medium larva (b), large larva (c) and pupae (d).



6. Acoustic detection system : An automated acoustic (Sound) detection system that counts the insects in a grain sample by analyzing the spatial and temporal distribution of sounds. The acoustic location-fixing insect detector is an automated system to quantify hidden infestation in 1 kg samples of wheat. It analysis input from an array of sensors embedded in the sample container walls. It identifies a specific pattern of input as an insect. The rate of sound detection is inversely proportion to weight. Thus, to reliably count insects with varying sound production patterns, the sound production identification needs to be calibrated.



7. Carbon dioxide method: The method is based on the fact that insects activity in the seed lot produces carbon dioxide (CO_2). By measuring the quantity of carbon dioxide produced by a given sample of seed in 24 h, the extent of internal infestation can be estimated.

< 0.3% CO_2	**=**	**insect-free seed sample**
0.3-0.5% CO_2	**=**	**slight infestation or m.c.>15%**
0.5-1.0% CO_2	**=**	**seed unfit for prolonged storage.**

This method not only requires a longer time, as high moisture content of seed interfere with the insect's respiration and its rate. It also fails to indicate the presence of dead insects inside the seed.

Pest infestation levels in seeds according to the different methods of instrumental analysis of CO_2 in 1 kg of material after 24 h incubation

Methods		
Gasometric (% CO_2 v/v)	**Infrared (ul of CO_2/min)**	**Level of infestation**
<0.2	<1.0	Nil or negligible
0.2	1.0	Low level
0.3–0.5	2.0–3.0	Light to moderate
0.6–0.9	4.0–6.0	Moderate to heavy
>1.0	>6.0	Heavy

8. Breeding out: Grain suspected of being infested may be incubated thus allowing insects to complete their life cycle.

The biggest disadvantage is the time factor since even under the optimum conditions of temperature and moisture content (26-30°C and 14-16% m.c.), at least 4-6 weeks will be required to breed out the full population of grain weevils and even longer incubation periods will be required for many other storage insect species

Conclusion :

Seed health testing against storage insects is important for seed quality assurance because insects affect both quality as well as quantity of stored seeds, besides good will in the market, seed crop health and grain production. The comparative evaluation of five detection techniques namely breeding out, CO_2 method, flotation method, ninhydrin method, and x-ray method of hidden infestation of *S. zeamais, R. dominica, S. cerealella, and C. chinensis* in cereals and pulses revealed the following order (from high to low) in terms of accuracy in detection: breeding out > ninhydrin method > x-ray method > flotation method, and the CO_2 method was rapid but not quantitative.

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Management of Seed Stores

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Seed storage management implies the maintenance of the harvested seed mass in good physical and physiological condition from the time of harvesting up to the time of their replanting. Seed ageing and loss of germination during storage cannot be checked altogether. However, it could be reduced appreciably by proper pre storage treatment to the product and providing good storage conditions. Seeds should be stored dry and kept dry. Seeds should be handled more like eggs than like stones. The period of time that seed can be stored without decline in viability is a function of their storage environmental variables and initial seed quality. The simplest and the oldest method of storage is to store dry seed in bags near air temperatures. This is termed as ambient storage or normal temperature storage. Many species can be stored in this way for a year or longer. Conditioned storage is necessary for longer periods and for extra sensitive seeds. Seed longevity in storage rooms depends upon a number of factors. The factors other than kind and variety of seeds are:

Factors Affecting Seed Longevity in Storage

Initial Seed Quality: Seed lots figuring high in initial seed quality store longer than deteriorated lots. The important implication of this is that only high quality seed should be carried over. The medium quality seed may be retained for the next planting season. The low quality seeds should be normally not considered for storage. Low quality seeds decline rapidly in storage. Initial seed quality reflects pre harvest history of the seed lot and the amount of care during the harvesting, transport, threshing, conveying and processing. Well maintained and adjusted post harvest handling equipment are essential for retaining the highest seed quality.

Moisture content: Life of seed and its span largely revolves around its moisture content and it is essential to dry seeds to safe moisture content. Over the moisture range of about 8 to 12%, the rate of seed deterioration increases as the moisture content increases. At higher moisture contents, the losses could be rapid due to mold growth and/or due to heating. Most seeds are good thermal insulators and, therefore they do not permit heat energy to transmit through them easily. Thermal resistance of wheat seed is considered 6 to 10 times higher than concrete. Minor source of heat in the form of moist seed may cause serious rise in its temperature and develop hot spots. Also, within the normal range, the biological activity of seeds, insect and mold further increase as the temperature increases. However, it is important to note that very low moisture content (< 4%) may also damage seeds due to extreme desiccation or cause hard seededness in some species.

Relative humidity and temperature during storage: Relative humidity and the temperature in the air of the seed storage room are the major environmental factors

influencing the storage life of the seeds. Low relative humidity makes the air thirsty of water and it picks up the unwanted moisture from the seed. Hence the seeds are kept dry in low humidity condition. Seeds achieve a rather specific and characteristics moisture content, termed as equilibrium moisture content, when subjected to a given combination of atmospheric relative humidity and the air temperature. This results due to the hygroscopic nature of the seeds. Fortunately, the establishment of moisture equilibrium in seeds is a time dependent process and it does not occur instantaneously. Therefore, the diurnal fluctuations in the relative humidity have little effect on moisture content.

Temperature also plays an important role in life of seed. Within the normal range, insect and molds increase as the temperature increases. Decreasing temperatures, relative humidity and moisture, therefore, is an effective means of maintaining seed quality in storage. Low temperature, low humidity storage of dry, cleaned and healthy seeds is the key to effective seed storage management.

Temperature Control:

Temperature is one of the most important environmental factors which influence seed storability. The lower the temperature, the longer the seed maintain good quality. Temperature control may be achieved by ventilation, insulation and refrigeration. These methods are not mutually exclusive and are used in combination.

Ventilation: Ventilation can be used to lower seed temperature and seed moisture control when used judiciously. Ventilation is suitable for minor downward adjustment of temperature (and to a lesser extent the moisture). It can also help to prevent hot spots from developing; the formation of convection air current; and maintenance of uniform seed moisture content and temperature. Right time of ventilation is when the outside temperature and relative humidity are low. At that time the exhaust fan can be put on.

Insulation: The walls, ceiling and floor of a seed storage room must have satisfactory heat insulation and a moisture vapor seal. Floor insulation is frequently installed in a bed of hot asphalt, which provides a good vapor seal. The types of material used may be fiberglass, spray-on-foam, Styrofoam, saw dust, glass wool cork etc. The insulation materials must be kept dry for maximum efficiency. The moisture protection must be provided outside the insulation, if the material does not have a characteristic for dryness naturally built into it. Board type insulation is applied in 2 or more layers. The joints are lapped and/or staggered to minimize heat and moisture penetration at joints. Ceiling insulation can be of many kinds. Ceiling and wall finishes usually consist of one half inch or more cement plaster applied as two coats. Wood, metal, or concrete bumpers are installed on walls where trucks and tractors might accidentally hit them. Low temperature seed storage rooms must have no windows and their doors must be well insulated and well sealed. For large openings, the roller-mounted door (siding door) may be preferred over swinging doors. A relatively novel idea is to use a high velocity stream of cool air across the inner face of the door. Double door air locks and small anterooms also help reduce heat and moisture entering low temperature low humidity seed storage rooms. Adequate measures for checking the leakage of heat and moisture can be provided at the time of planning and

building such seed stores. This job is better left to construction consultants and seed technologists should provide the functional requirements.

It is usually desirable to construct several low temperature rooms rather than a single large warehouse. In this ways annual operating costs can be lowered significantly. During the period when only small lots of seeds are stored, one or two rooms rather than the entire warehouse can be kept refrigerated. Most refrigerated seed storage facilities use forced air circulated through a cooling coil and then through the room. For large areas, a duct system distributes the cold air uniformly throughout the room.

Classification of moisture and heat removal systems configuration

System type	Components	Operation
I	Refrigeration compressor, motor and fans, evaporator and condenser coils	System is placed inside the conditioned space. Inside air is re-circulated through the unit until the set relative humidity is reached and the humidistat shut the unit off. It turns the unit on when the RH begins to rise due to product or system variables. Suitable where the sensible heat does not raise the temperatures above safe limits.
II	Desiccant, heater coils, conditioned air blower, and reactivation blower	Desiccant dehumidifier is located outside the conditioned space. Air in the conditioned space through a closed system, is re-circulated through, the unit until the set relative humidity is reached. A humidistat located inside the seed stores controls the running of the plant.
III	Conventional type split air conditioner	Evaporator section of the refrigeration unit is placed inside the conditioned space. Air is recirculated over the cold evaporator coil. Outside air is drawn over the condenser coils releasing the transferred heat to the atmosphere. A thermostat controls the unit. Electrical heater strips are sometimes used to add heat to the system for RH control.
IV	Desiccant dehumidifier with water after cooler	The water cooler reduces the air temperature as it leaves the desiccant dehumidifier. Effective for maintaining low humidities.
V	Refrigeration unit and the desiccant dehumidifier	Air in the conditioned space is cooled by pre-cooling coil before dehumidification. In the dehumidifier, latent heat of condensation is converted into sensible heat. Therefore, the after-cooling coil is provided. Pre-cooling and after-cooling is provided by refrigeration system.
VI	Refrigeration type dehumidifier and cooler	A self-contained refrigeration-type dehumidifier located inside the conditioned space removes the moisture from the air. The sensible heat load is handled by a refrigeration unit that transfers the heat to the outside atmosphere.
VII	Split air-refrigeration and desiccant dehumidifier	A dual system. The refrigeration system independently dehumidifies (within limits) and cools the air. The desiccant dehumidifier has much larger moisture extraction capacity. Offers a factor of safety in extreme conditions.

Refrigeration: Refrigeration is the household term. It is a process by which the heat is made to flow from lower to higher temperature, i.e., against the natural heat transfer process. It is the only method to achieve and maintain low temperature on long term basis. The medium employed to absorb heat is the refrigeration agent or simply refrigerant. Mechanical refrigeration systems are based on the ability of liquid heat as they vaporize. The vaporizing temperature of the liquid can be regulated by controlling the pressure at which the liquid vaporizes. In closed systems, the vapor is condensed back into liquid and thus used over and over again to provide a continuous flow of liquid for vaporization. Of

all the fluids currently used as refrigerant, the one nearest to idle general purpose refrigerant is refrigerant-12 or R-12. It has a saturation temperature of –29.8°C. It can be stored as a liquid at ordinary temperature only under pressure in heavy steel cylinders. A typical mechanical refrigeration system contains the following parts: (1) An evaporator to provide heat transfer surface through which heat moves from the space being refrigerated into the vaporizing refrigerant; (2) a suction line to convey the refrigerant vapor from the evaporator to the compressor; (3) a compressor to heat and compressor the vapor; (4) a hot gas or discharge line to carry the high-temperature, high-pressure vapor from the compressor to a condenser; (5) a condenser to provide heat transfer surface through which heat passes from the hot gas to the condensing medium; (6) a receiving tank to hold the liquid refrigerant for future use; (7) a liquid line to carry the liquid refrigerant from the receiving tank to the refrigerant metering device; and (8) a refrigerant metering device to control the flow of liquid to the evaporator. The typical vapor-compression system is divided into a low and a high-pressure side. The refrigerant metering device, evaporator, and suction line constitute the low pressure side of the system; the compressor, discharge line, condenser, receiving tank, and liquid line constitute the high pressure side of the system. A mechanical refrigeration system that will cool at a rate equivalent to melting one tone of ice in 24 hours is said to have a capacity of one tone refrigeration. The capacity of the compressor must be such that the vapor is drawn from the evaporator at the same rate at which it is produced.

Controlling Humidity: Relative humidity is measured by taking dry bulb and wet bulb temperature reading and finding the relative humidity from psychometric charts. Lower the wet bulb depression (dry bulb temperature – wet bulb temperature) lower is the relative humidity and vice-versa. At 100% relative humidity the wet bulb temperature and dry bulb temperature equalize. Humidity control systems are of two types. These are the refrigeration type and the desiccant type. The refrigeration type dehumidifier draws warm, moist air over a metal coil with fins spaced far enough apart to permit partial frosting and still allow for sufficient air passage. To be effect at low temperatures, a refrigeration type dehumidification system must cool the air below the desired temperature and reheat to the desired temperature. Air handling units are available with built-in refrigeration coils, electric defrosters, and reheat coils. Dehumidifiers using liquid or solid desiccants in conjunction with refrigeration can frequently reduce the cost of maintaining very low relative humilities. The dehumidifier incorporates one or two beds of granulated silica gel or activated alumina, which can absorb much water vapour. Now a day the rotary bed dehumidifiers are in practice. The rotary bed dehumidifiers have one or more beds divided into two air streams. The bed rotates slowly, and while part of each bed is absorbing water vapor from the air stream, the remainder is being recharged.

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Determination of Moisture Content of Seed Lots

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Testing moisture content in Seed Sample is very important aspect of Seed Testing. The moisture content of a sample is the loss in weight when it is dried in accordance with the Seed Testing Rules. It is expressed as a percentage of the weight of the original sample. The submitted sample shall be accepted for moisture determination only if it is in intact, moisture - proof container from which as much air as possible has been excluded. The moisture determination of the seed sample must be started at earliest possible after receipt of sample.

Procedures:

Weighing shall be in grams to three decimal places. Seeds of larger size (Table 1.) are ground before drying unless its high oil content makes it difficult. After grinding, the sample is passed through different sizes of sieves (Table 2.). Pre-drying before grinding is required for samples having moisture content more than 17%. After pre-drying, the sub-samples are reweighed in their containers to determine the loss in weight.

I. Low constant temperature oven method:

The working sample must be evenly distributed over the surface of the container. Weigh the container and its cover before and after filling. Place the container rapidly, on top of its cover, in an oven maintained at a temperature of 103 ± 2°C and dry for 17 ± 1 hour. The drying period begins at the time the oven returns to the required temperature. At the end of the prescribed period, cover the container and place it in a desiccators to cool for 30-40 minutes. After cooling, weigh the container with its cover and contents. The relative humidity of the ambient air in the laboratory must be less than 70% at the time of final weighing. ISTA prescribes the low constant temperature oven method, for all tree species. Normally oilseeds are subjected to low constant temperature oven method while cereals and pulses are subjected to high constant temperature oven method.

II. High constant temperature oven method:

The procedure is the same as low constant temperature oven method, except that the oven is maintained at a temperature of 130-133°C, the sample is dried for a period of four hours for tree species and no special requirement pertain to the relative humidity of the ambient air in the laboratory during determination.

The moisture content expressed as a percentage by weight shall be calculated to one decimal place by means of the following formula:

$$\% = \frac{M2 - M3}{M2 - M1} * 100$$

Where,

M_1 - is the weight of the container and its cover (in grams),

M_2 - is the weight of the container & its cover (in grams) and its contents before drying and

M_3 - is the weight of the container, cover (in grams) and contents after drying.

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

$$S1 + S2 - \frac{S1 * S2}{100}$$

Table 1. Grinding requirements for Different Crop Seeds:

Crop	**Grinding**	**Mesh size**	
Paddy, wheat, maize, sorghum, cotton	Fine	50% ground material is passed through 0.5 mm mesh	10% ground material remain on 1.00 mm mesh
Pea, chickpea, soybean, lathyrus	Coarse	50% ground material is passed through 4 mm mesh	

Table 2 . Pre-drying requirements:

Crop	Moisture content	Temperature required for Drying (in °C)	Duration
Maize	>25%	>0	2 - 5 hrs
Rice	>13%	130	5 - 10 min
Soybean	>10%	130	5 - 10 min

III. Universal moisture meter:

Universal moisture meter is a popular and most dependable instrument for moisture estimation. The following are its essential parts:

1. Compression unit
2. Moisture meter dial
3. Thermometer
4. Compression knob
5. Cups of different volumes

Moisture estimation is made quick by the advent of digital moisture meters. The principle involved is that electrical conductivity of moist material is directly proportionate to the amount of moisture content in it. A representative sample of prescribed weight or volume (Table 3.) is taken and placed in the sample cup. It is fixed in the lower house of compression unit.

Meter is calibrated by pressing the button "CAL" and "BELL" with the help of calibration knob. Sample is compressed as per requirement with the help of compression knob and scale. At required compression the meter dial (M) is read by pressing the knob

"Read" and bell. Temperature (T) is observed by the thermometer fixed in between meter dial and compression chamber. The reading M and T are intercepted on the corelator dial (moisture meter dial) by turning the temperature dial. On adjustment of both the reading mark of arrow on the outer reading of temperature dial indicates the moisture percentage. For some crops factor is also considered for estimation of moisture content.

Table 3. Determination of moisture content by universal moisture meter:

Crop	Sample size		Compression	Factor
	Weight (g)	Volume*		
	FIELD AND FODDER CROPS			
Barley	50	B	0.600	
Maize	60	B	0.560	
Oat	30	B	0.400	
Pearl millet	60	B	0.500	
Rice	50	B	0.550	
Sorghum	50	B	0.675	
Wheat	30	A	0.275	Add 1%
Moong and urid		A	0.275	Add 1.5%
Chickpea		C	0.500	Subtract 1%
Horse gram		A	0.275	
Lentil		A	0.250	x 0.7 + 3.5%
Pigeon pea, field pea		C	0.450	
Castor		C	0.500	Multiplied by 0.5
Groundnut	25		0.300	Multiplied by 0.6
Groundnut (kernel)	26		0.450	Multiplied by 0.56
Safflower	15		0.450	Multiplied by 0.66
Sesame			0.550	Subtract 0.5%
Soybean	60	C	0.575	Subtract 2.5%
Sunflower	30	B	0.500	Multiplied by 0.6
Rape seed and mustard			0.450	Multiplied by 0.6
Cotton (linted)	30	C	0.360	Subtract 5%
	VEGETABLES			
Kidney bean	50	B	0.400	
Okra		C	0.425	
Cabbage		A	0.260	Multiplied by 0.6
Cowpea		A	0.325	Multiplied by 0.8
Cucumber		B	0.525	Multiplied by 0.8
Lettuce		B	0.500	Multiplied by 0.9
Onion		A	0.250	Subtract 2.5%
Tomato	25	B	0.250	Multiplied by 0.8
Turnip	25		0.200	Multiplied 0.8
Watermelon		B	0.425	Subtract 3.5%

Coriander		C	0.325	Multiplied by 0.6

* A, B and C - Container size

The moisture content must be reported to the nearest 0.1% in the space provided on the Analysis Certificate. Seed lot with moisture content more than the minimum seed certification standards (Table 4.) are recommended for drying.

Table 4. Minimum seed certification standard for moisture percentage:

Crop	Sample in vapour proof container	Sample not in vapour proof bag
FIELD AND FODDER CROPS		
Castor, mustard, taramira	5	8
Groundnut, niger, sesame	5	9
Cotton	6	10
Rape seed	7	8
Linseed, horse gram, rajmash, safflower, sunflower, jute	7	9
Berseem, lucerne, Indian clover	7	10
Soybean	7	12
Moong, urid, chickpea, field pea, pigeon pea, lentil, lathyrus, kidney bean, rice bean	8	9
Buffel, Dharaf, Dinanath, guinea, marvel, setaria and stylo grass	8	10
Wheat, maize, sorghum, pearl millet, barley, triticale, oat, minor millets, teosinte, forage sorghum	8	12
Rice	8	13
VEGETABLES		
Rat tail radish, radish, turnip	5	6
Cole crops	5	7
All cucurbits	6	7
TPS, brinjal, tomato, chilli, capsicum, onion, fenugreek, lettuce, amaranth, asparagus	6	8
Carrot, celery, parsley	7	8
French bean	7	9
Cowpea, Indian bean, cluster bean, spinach, sugar beat	8	9
Okra	8	10

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Determination of Physical Purity of Seed Lots

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Quality seed is the basic need to any farmer to realize the benefit of all other inputs provided by him to raise any crop. Seed quality includes genetic purity, physical purity, germination percent, vigour and optimum moisture content *etc.* Any public or private sector which are producing and marking the seed should ensure that the seed reaching the farmer must have all the above qualities within it. Nowadays with the advancement of science, there are lot of new techniques are employed to assure the quality of seed. To assess the seed quality we are following International Seed Testing Association (ISTA) rules for international trade and National rules for seed law enforcement in our country. In this chapter we are going to discuss about the analysis of physical purity of seed samples.
The physical purity analysis of a seed sample in the seed testing laboratory refers to the determination of the different components of the physical purity *viz*., pure seed, other crop seeds, weed seeds and the inert matter (as per national rule). According to ISTA rule the physical purity components are pure seed, other seed and inert matter.

Objective of the purity analysis

The objective of the purity analysis is to determine whether the submitted seed sample (by inference the seed lot) conforms to the prescribed quality standards in regard to purity components.

Method of physical purity analysis

The physical purity analysis should be carried out on working samples of prescribed weight (Table.1) and the percentage of different component is determined by weight.

Table.1. Submitted and working sample size for some of the important crop

Sl. No.	Crop	Submitted sample size (g)	Working sample for purity analysis (g)
1	Paddy	400	40
2	Wheat	1000	120
3	Maize	1000	900
4	Sorghum	900	90
5	Pearl millet	150	15
6	Pigeon pea	1000	300
7	Chick pea	1000	1000
8	Green gram	1000	120
9	Black gram	1000	150
10	cowpea	1000	400
11	Pea	1000	900
12	Peanut	1000	1000, 600(kenels)
13	Brinjal, Chilli and	150	15

	Sweet pepper		
14	Tomato (Variety) Tomato (Hybrid)	70 7	7 7
15	Okra	1000	140
16	Cucumber	150	70
17	Bitter gourd	1000	450
18	Ash gourd, bottle gourd	700	70
19	Cabbage, cauliflower	100	10
20	Cotton variety (linted) Cotton variety (delinted) Cotton hybrid (linted) Cotton hybrid (delinted)	1000 350 350 250	350 35 35 25

The number of decimal places to which the working sample and the components of the working sample should be weighed is given below.

Weight in gram	Number of decimal places	Example
Less than one	4	0.9525
1-9.999	3	9.525
10-99.99	2	95.25
100-999.9	1	952.5
1000 or more	0	9525

Equipment / Apparatus for purity analysis: The apparatus necessary for purity analysis are Seed dividers, purity table, hand lens, electronic balance (1 mg), seed herbarium of crop and weed seed, stereoscopic binocular, seed blower, sieve, Diaphanoscope, sample pans, purity dish, forceps and spatula. Seed scanner, digital microscope camera, LED transmitted light mounted microscope, blower with monitoring and calibration facility, continuously flowing blower and ergovision system are some of the advanced equipment used for purity analysis of seed (Garay, 2012).

Seed scanner

Seed scanner (fig.1) mainly used for cereals. 30 samples can be loaded at a time. It will separate good quality and questionable seed.

Fig.1 Seed scanner

Digital microscope camera: Here the digital microscope camera (fig.2) attached with the computer. The purity analysis can be done with more convenient manner.

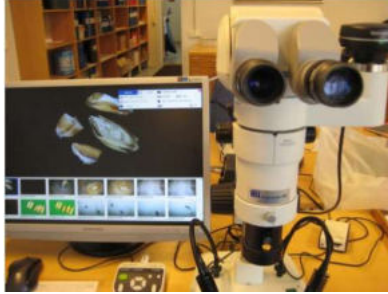


Fig.2 Digital microscope camera

LED transmitted light mounted microscope: LED transmitted light mounted microscope provide a clear visibility of seeds and favour perfect examination of seeds (fig.3)

Fig.3. (a). LED tra in microscope with light above, (c). As seen in microscope with LED transmitted light from below

Blower with monitoring and calibration facility: The procedure followed by AOSA will be adopted by ISTA with respect to utilization of blower with monitoring and calibration

facility (fig.4). Uniform separation of pure seed and inert matter of calibration sample was achieved by air velocity calibration method.

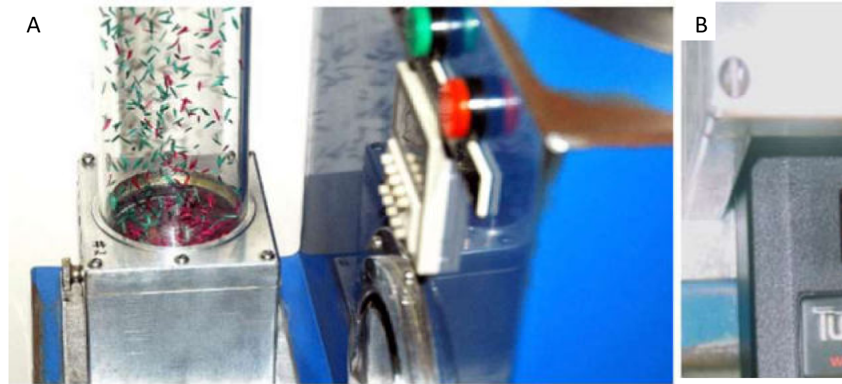


Fig.4. a) Use of Material Collection system to calibrate a blower, b).air velocity monitoring in blower

Continuous Flowing blower: Continuously flowing blower (fig.5) can separate the sample in to three fractions (fig.6). It is designed with built in anemometer. Air velocity can be calibrated. Any size of seed and large sample can be analysed.

a. Light weight, immature and diseased

b. Medium weight seed

c. Heavy weight selected

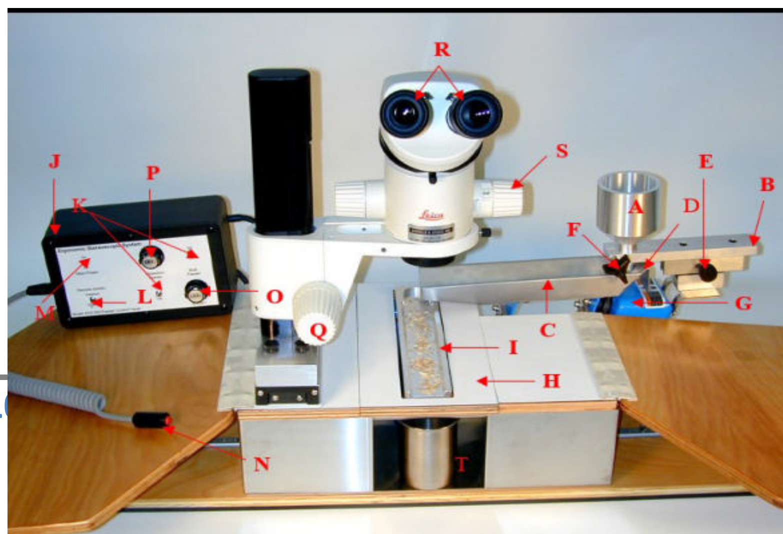
Fig.5 Continuous flowing blower

Fig.6. Clover seeds separated by Continuous flowing blower

Ergo vision system: Oregon State University seed laboratory is designed ergovision system for purity analysis of seed. It is highly comfortable system that integrates ergonomics, continuous seed flow, a choice of optical system, precise feeder controls, interchangeable inspection trays and seed hoppers, and a hand or foot switch to stop and start the vibratory feeders. In addition, the whole microscope mount can be adjusted back and forth, and the eye piece can be adjusted to the need of the operator (Garay *et al*., 2010).

Operation of Ergo vision system

1. The working sample is place in the sample holding funnels and inspection trays of different sizes can be used to accommodate seeds of different sizes. The seed flows from the funnel to trays that are calibrated to the desired level.
2. The feeder tray moves the seeds to the inspection tray where the seeds are inspected. The speed of seed flow can be controlled by adjusting the vibration of the seed tray as desired by the analyst. The inspection trays are designed to spread the seeds uniformly. They are interchangeable, so that very small seeds such bentgrass (Agrostis spp.) to large seeded species such as wheat stay within the field of view.
3. The seeds are examined using a high quality microscope, Mantis Inspection Viewer or video camera. The magnification can be adjusted at will, depending on the kind of seed and the kind of contamination being inspected.
4. The image clarity can be enhanced by fibre optic or LED lighting directed to the viewing area.
5. The flow of seed can be stopped at any time to make a closer examination of any object and to separate the contamination from the sample.
6. The inspected seeds are automatically deposited from the inspection tray into the sample holding cup in the front of the inspection station.



A. Funnel
B. Funnel holder plate
C. Bulk feeder tray
D. Back plate of bulk feeder tray
E. Funnel adjustment knob
F. Funnel clamp knob
G. Bulk feeder vibrator
H. Removable cover
I. Inspection tray
J. Feeder control panel
K. Feeder switches
L. Remote switch
M. Main power switch
N. Hand switch
O. Bulk speed dial
P. Inspection speed dial
Q. Main focus knob
R. Eye piece focus
S. Magnification setting

Making the purity separation: Purity table should be cleaned. The working sample should be sepated in to different component with help of sieve, blower or any other appropriate apparatus. Each particles should be examined individually based on its size, shape, colour and surface structure. The impurities like other crop seed (OCS), weed seed and inert matter should be separated and kept separately. Pure seed should be collected in separate pan. OCS and weed should be identified and recorded in analysis card. All the component should be weighed in gram and recorded. The percentage of each component calculated based on weight. A retest has to be conducted, if the initial and final weight varies more than 5 per cent.

Definitions of purity components

Pure seed: The pure seed means the seeds of kind/species stated by the sender, or found to predominate in the test. It includes all botanical varieties and varieties of that kind/species.
The following structures (even if immature, undersized, shrivelled, diseased or germinated, provided they can definitely be identified as of that species) are regarded as pure seed, unless transformed into fungal sclerotia, smut balls or nematode galls.
(a) Intact seed units or pod in case of groundnut, i.e. seeds in the botanical sense; achenes and similar fruits, schizocarps and mericarps with or without perianth, and regardless of whether they contain a true seed or not, unless it is readily apparent that no true seed is present. In case of *Graminae* the florets with an obvious caryopsis containing endosperm and free caryopses.
(b) Pieces of seed units or pod in case of goundnut larger than one-half the original size.

Exceptions
From the above main principles certain exceptions are made for particular genera or species.
a. Seed units of the families *Leguminosae, Cruciferae* with the seed coats entirely removed are regarded as inert matter
b. In *Beta* (except genetic monogerm cultivars) seed units larger than certain size are classified as pure seed as elaborated under *Chelnopodiaceae*.
c. In *Gramincae* following rules are to be followed for the certain genera mentioned below.

1. A minimum size of the caryopsis is required in the case of *Lolillm, Festuca* and *Agropyron*. A floret with a caryopsis one- third or more of the length of the palea measured from the base or the rechilla is regarded as pure seed or other seed. But a floret with a caryopsis less than one third the length or palea is regarded as inert matter.
2. Attached sterile florets are not removed in the case of *Avena, Dactylis, Poa* and *Sorghum* and left attached and included in the pure seed fraction.
This rule applies also to the attached sterile florets of *Lolium* which do not extend to the tip of sterile floret, excluding the awn
3. The presence of caryopsis in spikelets and florets is not always obligatory in genera specified in the pure seed definitions.
4.'I'he separation of pure seed and inert matter is done by a uniform blowing method.
5. Multiple units are left in the pure seed fraction as in *Dactylis* and *Festuca*
The following structures are classified as multiple seed units
- One fertile floret with one fertile or sterile floret attached that extends to or beyond the tip of the fertile floret, excluding the awns,
- One fertile floret with more than one attached fertile and/or sterile floret of any length
- One fertile floret with basally attached floret of any length

Florets with attached single fertile or sterile floret that does not extend to the tip of the fertile floret, excluding the awn, are treated as single seed unit. The attached fertile or sterile floret is not removed.

Pure seed definitions applicable to specific genera
Gramineae

1. *Cynodon, Dechampsia*
Caryopsis, pieces of caryopsis larger than one-half the original size.
Floret with lemma and palea enclosing, caryopsis with or without awn.

2. Phalaris
Caryopsis, pieces of caryopsis larger than one-half the original size.
Floret with lemma and palea enclosing a caryopsis, plus attached sterile lemmas with or without awn including protruding anther, if present.

3. Hordeum
Caryopsis, pieces of caryopsis larger than one-half the original size.
Floret with lemma and palea enclosing a caryopsis, excluding entire awn when the length of the awn is longer than the length of the floret.

4. Brachiaria, Echinoclea, Milinis, Panicum, Paspalum, Setaria
Caryopsis, pieces of caryopsis larger than one-half the original size.
Spikelet with glumes, lemma and palea enclosing a caryopsis, plus attached sterile lemma (with or without awn in the case of *Echinoclea* and *Milinis*).
Floret with lemma and palea enclosing a caryopsis.

5. Oryza

Caryopsis, pieces of caryopsis larger than one-half the original size.

Spikelet with glume, lemma and palea enclosing a caryopsis, excluding entire awn when the length of the awn is longer than the length of the floret.

Floret with lemma and palea enclosing a caryopsis, excluding entire awn when the length of the awn is longer than the length of the floret.

6. Secale, Triticosecale, Triticum, Zea

Caryopsis, pieces of caryopsis larger than one-half the original size.

7. Avena

Caryopsis, pieces of caryopsis larger than one-half the original size.

Spikelet with lemma and paleae including caryopsis with or without awn, plus attached sterile floret.

Separate spikelets consisting of two fertile florets, (do not separate units where the lemma of the outer floret partly envelops the inner fertile floret), remove stalk at the point of attachment (Single florets containing only the ovary are classed as inert matter).

Floret, with lemma and palea enclosing a caryopsis, with or without awn.

8. Cenchrus, Pennisetum

Caryopsis, piece of caryopsis larger than one-half the original size.

Fascicle of 1-5 spikelets (spikelet with glumes, lemma and palea enclosing a caryopsis plus attached sterile lemma) with involucre of bristles.

Floret with lemma and palca enclosing a caryopsis (In *cenchrus* : spikelet and floret with or without caryopsis).

9. Dichanthium

Caryopsis, piece of caryopsis larger than one-half the original size.

Fertile (sessile) spikelet (with glumes, lemma and palea enclosing a caryopsis, with or without awn, plus attached sterile lemma, with attached pedicel (of sterile spikelet) and rachis segment.

10. Sorghum

Caryopsis, piece of caryopsis larger than one-half the original size.

Fertile (sessile) spikelet (consists of hardened glumes enclosing two florets (therefore not visible), one consisting of a transparent sterile lemma the other containing the caryopsis within a tissue-like lemma and palea, with or without awn) with attached pedicel (of sterile spikelet) and rachis segment (stalk), provided that the stalk is no longer than the spikelet, when the stalk is longer than the spikelet the entire stalk is to be removed.

Leguminosae**:** Seed, provided a portion of the testa is attached.

Exceptions: Piece of seed larger than one-half the original size, provided a portion of the testa is attached.

Lespedeza**:** also the pod, with or without calyx or bracts, with one seed.

Stylosanthes**:** also the pod, with or without seed unless it is obvious that no seed is present.

Pedaliaecae, Scrophillariaceac, Convolvulaceae, Papavaraceae, Linaceae, Caryophyllaceae, Tiliaceae, Amarathaceae, Solanaceae, Cucurbitaceae, Liliaceae**.**

Seed with or without seed coat.

Piece of seed larger than one-half the original size, with or without testa.

Compositae**:** Seed with or without testa, piece of seed larger than one-half the original size with or without testa.

Carthamus, Chichorium, Helianthus, Lactuca

Achene, with or without beak, or with or without pappus, unless it is obvious that no seed is present.

Piece of achene larger than one-half the original size, unless it is obvious that no seed is present.

Seed, with pericarp/testa partially or entirely removed.

Piece of seed larger than one-half the original size, with the pericarp/testa partially or entirely removed.

Carum

Schizocarp/mericarp, with or without pedicel (of any length of frequency), unless it is obvious that no seeds are present.

Pieces of mericarp larger than one-half the original size, unless it is obvious that no seed is present.

Eruca

Seed, with the pericarp partially or entirely removed.

Piece of seed larger than one-half the original size, with the pericarp partially or entirely removed.

Cruciferae

Raphanus**:** One-seeded segment of pod or silique, with or without stalk or terminal beak, Seed, provided a portion of testa is attached.

Piece of seed larger than one-half the original size, provided a portion of testa is attached.

Sinapsis, Brassica, Lepidium

Seed provided a portion of the testa is attached. Piece of seed larger than one-half the original size, provided a portion of the testa is attached.

Malvaceae

Gossypium

Seed with or without testa.

Piece of seed larger than one-half the original size, with or without testa.

(NB: testa with or without hairs).

Hibiscus

Seed with or without testa.

Piece of seed larger than one-half the original size, with or without testa.

Umbelliferae (*Petroselinum, Apium, Coriandrum, Cuminum, Daucus, Pastinaca*)

Schizocarp/mericarp, with or without pedicel (of any length of frequency,) unless it is obvious that no seeds are present.Pieces of mericarp lager than one-half the original size, unless it is obvious that no seed is present. Seed with the pericarp partially or entirely removed. Piece of seed larger than one-half the original size, with the pericarp partially or entirely removed.

Euphorbiaceae (*Ricinus*)

Seed, with or without tesla, with or without strophiole/caruncle.

Piece of seed larger than one-half the original size, with or without testa.

Chenopodiaceae

Beta

Cluster or piece of cluster, including attached stalk pieces (protruding stalk pieces that are longer than the width of the cluster, are to be entirely removed) retained on a 200 mm x 300 mm rectangular sieve, with square ended slots1.5mm x20 mm when shaken for one minute, with or without seed.

Genetic monogerm cultivars only

Cluster, or piece of cluster, including attached stalk pieces (protruding stalk pieces that are longer than the width of the cluster are to be entirely removed) unless it is obvious that no seed is present.

Seed, with the pericarp/testa partially or entirely removed.

Piece of seed larger than one-half the original size with the pericarp/testa partially or entirely removed.

Spinacea

Achene, unless it is obvious that no seed is present. Piece of achene larger than one half the original size, unless it is obvious that no seed is present.

Seed, with pericarp/tesla partially or entirely removed. Piece of seed larger than one-half the original size with the pericarp/testa partially or entirely removed.

Cannabaceae

Cannabis

Achene, unless it is obvious that no seed is present.Piece of achene larger than one-half the original size, unless it is obvious that no seed is present.

Seed, with pericarp/testa partially or entirely removed. Piece of seed larger than one-half the original size with the pericarp/testa partially or entirely removed

Other Crop Seed

It relates to the kinds of crops other than the kind being examined.

With respect to classification as other crop seeds the distinguishable characteristics described under pure seed shall also be applicable.

Weed Seed

It includes seeds of those species normally recognised as weeds or specified under a regulation of the Seed Act as a noxious weed. For classification, distinguishable characteristics set out for pure seed shall be applicable to weed seed.

Exceptions

1. Beta seed units are evaluated without sieving using the pure seed definition for monogerm cultivars.
2. Seed units of species for which a uniform blowing procedure applies are evaluated without blowing.
3. Multiple seed units shall be separated and the single units classified according to the general principles.
4. *Cuscuta* spp. seed units which are fragile and ashen grey to creamy white in colour are classified as inert matter.

Multiple structures, capsules, pods are opened and the seeds removed and the non-seed material placed in the inert matter, except for certain species or genera as indicated in the pure seed definitions.

Inert Matter

Inert matter includes seed units and all other matter and structures that are not defined as pure seed, other crop seed or seed as follows.

1. Seed units in which it is readily apparent that no true seed is apparent.
2. Beta seed units (except genetic monogerm varieties) passed through a 200 mm x 300 mm rectangular sieve with square ended slots 1.5 mm x20 mm when shaken for one minute, with or with one seed.
3. Florets of *Lolium, Festuca, Agrypyron* with a caryopsis less than one-third the length of palea is regarded as inert matter.
4. Sterile florets attached to a fertile floret are to be removed except in *Arrhenotherum*, *Avena, Chloris, Dactylis, Festuca, Holcus, Poa and Sorghum* are attached sterile florets of *Lolium* which do not extend to the tip of the fertile floret, excluding the awn.
5. Pieces of broken or damaged seed units half or less than half the original size.
6. Those appendages not classed as being part of pure seed in the pure seed definitions must be removed and included in the inert matter.
7. Seed of *Leguminosae, Cruciferae, Cupressaceae, Pinaceae and Taxodiaceae* with the seed coat entirely removed.
8. Seeds of *Cuscuta* species which are fragile or ashen grey to creamy white in colour.
9. Unattached sterile florets, empty glumes, lemmas, paleas, chaff, stems, leaves, cone scales, wings, bark, flowers, nematode galls, fungus bodies such as ergot, sclerotia and smut balls, soil, sand, stones and all other non-seed matter.
10. Awns, stalks longer then florets, spikelets are to be removed and treated as inert matter.
11. All material left in the light fraction when the separation is made by the uniform blowing method except other seeds.

In the heavy fraction, broken florets and caryopsis half or less than half the original size, and all other matter except pure seed and other seed (Agrawal, 1980).

Reporting of results

Percentage of each component should be reported with one decimal place. The percentage should be calculated based on sum of weight of all component not based on original working sample weigh. If the component is nil, it has to be reported as "0.0". If the component is 0.05-0.1%, than it should be reported as "trace". The name of the each crop

species should be mentioned with Latin name. if it is difficult at least genus has to mentioned in report.

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Role and importance of seed testing in seed quality control

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A seed is an embryonic plant and a store of food that is encased in a protective shell called a seed coat. The term "seed" is used more broadly to refer to anything that can be sowed, including seed and husk or tuber. After the embryo sac is fertilized by sperm from pollen, creating a zygote, the ripened ovule produces seeds. A seed's embryo develops from the zygote and expands within the mother plant until it reaches a particular size before growth is stopped. The ovule's exterior integument is where the seed coat originates.

The most typical method of plant reproduction is by seeds. Wind, water, animals, or other factors distribute them across geographical locations. A seed will germinate and grow into a new plant when it falls into an environment that is favourable for it to germinate.

The basic parts of a seed:

1. Embryo: The future plant is in the embryo. A root, a stem, and one or more leaves make up this plant.

2. Endosperm: The embryo's nutritional supply is stored in the endosperm. Starch, oil, and protein are the main ingredients of the endosperm.

3. Seed coat: The embryo's outermost protective covering is called the seed coat. It helps in protecting the embryo from its surroundings and deleterious factors present therein.

Depending on the plant species, seeds come in a wide range of sizes, shapes, and colours/shades. The seeds of orchids are among the tiniest ones, whereas the seeds of coconuts are among the largest of the seeds. Some seeds are smooth in texture, whereas others have a surface that is textured or lumpy. Some seeds have vivid colours, while others have dull ones.

Seed is an essential link in the food chain without any doubt. Birds, mammals, and insects all consume them for their health and survival. Humans, however, have also discovered the use seeds for other things, such as food and medicine.

Role of seeds:

Here are a few of the critical functions that seeds are known to play.:

1. Reproduction: Plants reproduce through the production of seeds.

2. Food: A variety of species, including humans, eat seeds as their principal food item. Seeds may be those of cereals, pulses, oilseeds and others.

3. Medicine: Some seeds have therapeutic qualities. For instance, flaxseed oil, a good source of omega-3 fatty acids, is produced from the seeds of the linseed plant.

4. Industry: Many industrial items, including polymers, lubricants, and cosmetics, include seeds.

5. Environmental conservation: Seeds play an important role in the conservation of the ecology, and sustainability of the environment. They are used to rebuild the damaged ecosystems and develop fresh habitats for living things.

Seeds, therefore, are a crucial component of the natural world. They are essential to the ecosystem, the food chain, and plant reproduction.

Seed testing and seed quality control:

Seed testing is the process of determining a seed's quality. It is a crucial component of the system used to ensure that seeds fulfil quality requirements in the seed quality control process. Seed testing is done to check a seed's germination potential, purity, moisture content, pest and disease-free status and other aspects that have a direct and/or indirect effects on the planting value of seeds.

The words seed testing and seed quality control are similar and frequently used interchangeably. But there is a slight distinction between the two. The total process of ensuring that seeds fulfil specific quality criteria is known as seed quality control. In addition to other tasks including seed production, handling, and storage, it also includes seed testing.

In other words, seed quality control includes seed testing. It is one of the tools used to make sure seeds meet particular quality requirements.

Purpose(s) of seed testing:

Seed testing serves the following purposes in seed quality control:

a. Make sure the seeds are of a high quality and will germinate successfully.
b. Stop farmers from purchasing seedsof inferior or dubious quality.
c. Stop diseases and pests from spreading in the environment.
d. Encourage fair seed trade.
e. Aid in increasing crop yields and ensuring food security.

2.

Importance of Seed testing:

The importance of seed testing cannot be overstated. To enumerate a few:

1. It first makes sure that the seeds are of a high quality and will germinate successfully. Given that germination is the process by which seeds turn into plants, this is significant. Farmers will not receive good produce if seeds do not germinate well.
2. Seed testing safeguards farmers from purchases of inferior quality seed. Poor-quality seeds may not germinate successfully or they may contain pests or pathogens. Crop losses and financial losses for farmers may result from this.
3. Seed testing provides protection against the spread of insect-pests and pathogens in the environment. Insect-pests and pathogens that infect seeds can then spread to other plants. Seed testing enables the detection and eradication of diseased seeds, aiding in environmental protection.
4. Seed testing is an integral part of ethics in seed trade. Fair seed trade ensures fair compensation to the farmers for the seeds they produce on their farms. By seed testing, farmers may avoid selling substandardseeds that would lower the value of their produce.
5. Crop yields and food security are improved with the help of seed testing. Increased agricultural yields from high-quality seeds can contribute to better food security. Having access to high-quality seeds for farmers is made possible with the aid of seed testing.

TESTING SEEDS FOR QUALITY:

It is possible to test seeds using a variety of techniques. The most popular techniques include disease testing, germination testing, purity testing, and moisture content testing.

1. TESTING FORGERMINATION:

The proportion of seeds in a seed lot that will germinate is calculated using germination tests. In order to do this, seeds are planted in a controlled environment, and the proportion of sown seeds is then calculated. In order to assess the quality of seeds, germination testing counts the proportion of seeds that will germinate under specific circumstances. To verify that seeds are of high quality and will produce successful germination, it is the most popular method of seed testing.

By sowing seeds in a controlled setting and tracking their germination over time, germination testing is performed. To guarantee precise and repeatable findings, there is a need for standardization of the conditions for the germination test. The following conditions are used for germination tests:

***1. Temperature*:**The temperature is recommended to be between 20-25 degrees Celsius (ºC)

2. Light: While not necessary for all seeds, light is often provided for the majority of the experiments.

3. Moisture: The germination medium's moisture content is maintained at a consistent level.

4. Duration: Typically, the germination test lasts 7 to 10 days.

The germination percentage is the proportion of seeds that germinate. High-quality seeds with good germination rates are indicated by a high germination percentage. Low germination rates are a sign of poor seed quality and potential poor germination. Germination testing is an essential component of seed quality management. It aids in ensuring that seeds are of a high quality and will germinate successfully to give an optimum plant stand in the sown area. The environment, farmers, and the world's food supply all benefit from this.

Factors affecting seed germination:

The following are some factors that may influence a seed's germination:

1. ***Age of seeds***: Age of the seed is an important factor that affects its germinability. The likelihood of germination decreases with seed age.
2. ***Moisture content***: Dry seeds seldom germinate. Moisture is needed to wash off the leachates responsible for seed dormancy. Seeds, therefore,require moisture in order to germinate.
3. ***Temperature***: Temperature is a good determinant of seed germination. At a particular temperature range, seeds germinate most effectively.
4. ***Light***: Light is required for the germination of some seeds but not others.
5. ***Diseases and pests***: Diseases and pest infestations might cause seeds to germinate with lower percentages.

2.

Testing seeds for Germination

The steps below can be used to perform a germination test:

1. Preparation of the germination media. This can be paper towels, soil mixture, or sand.
2. Sowing of the seeds in the germination medium.
3. Placing the germination medium with seeds sown in a controlled environment.
4. Monitoring the germination of the seeds periodicallyuntil they germinate or until the standard germination period of the species.
5. Calculation of the germination in percentage.

Seed Testing for germination is a clear and easy technique, but it is important to follow the right steps to get reliable findings.

Advantages of Germination testing:

The following are some advantages of germination testing:

1. It guarantees that seeds are of a high quality and will germinate successfully.
2. It helps farmers against purchasing seeds of inferior or dubious quality.
3. It provides protection against the spread of illnesses and pests in the environment.
4. It encourages seed fair trading.
5. Crop productivity and food security are improved.

(B) TESTING FOR PURITY:

It is employed to estimate the proportion of pure seeds present in a sample. To achieve this, the seeds are divided into many groups, such as crop varieties, weed seeds, and other crop species. Seed quality testing involves calculating the proportion of pure seeds in a sample to assess the quality of the seeds. In order to make sure that seeds are of excellent quality and will produce good germination, it is a crucial component of seed quality management.

Sorting the seeds into multiple categories, such as the crop type, weed seeds, and other crop species, is how seed purity testing is carried out. Then the percentage of pure seeds is determined. The quality of the seeds is indicated by the pure seed content, which should be high. The quality of the seeds and their potential for poor germination are indicated by a low percentage of pure seeds.

Components of seed purity testing:

The three primary components of seeds in a purity test are as follows:

1. Pure seed:The seeds for the type of crop that the sender specified. All botanical variations of that kind or species are included.

2. Other crop seeds:The seeds of unrelated crop kinds to the one that is specifically mentioned.

3. Weed seeds:Any other plant species' seeds, including weeds, grasses, and different kinds of crops.

Steps for seed purity testing:

The following steps can be used for seed purity testing.

1. ***Prepare some seeds as a sample:*** The sample size needs to be sufficient to serve as an accurate representation of the complete batch of seeds.
2. ***Sort the seeds into various groups*:** Either a machine or a person can carry out this task.
3. ***Determine the percentage of seeds that are pure:*** To do this, multiply the result of dividing the weight of the pure seeds by the weight of the entire sample by 100 to find the seed purity in percentage.

Although testing for seed purity is a simple and straightforward process, it is crucial to follow the right steps to achieve accuracy in results.

Advantages of seed purity testing:

The following are a few advantages of seed purity testing:

1. It guarantees that seeds are of a high quality and will germinate successfully.
2. It protects farmers against purchasing seeds of inferior quality.
3. It protects against the spread of weeds in the environment.
4. It encourages fair trading ofseeds.
5. Crop productivity and food security are improved.

2.

(C) TESTINGFOR SEED MOISTURE CONTENT:

It is done to estimate the moisture content of seeds. This is crucial since seeds will not germinate successfully in conditions that are either too humid or too dry. By calculating the amount of moisture in a sample, seed testing for moisture content evaluates the quality of seeds. In order to make sure that seeds are of excellent quality and will produce desired level of germination, it is a crucial component of seed quality management.

The moisture content of seeds is significant since it has an impact on how well they germinate. The right amount of moisture is necessary for seeds to germinate, but too much moisture will enhance the growth of fungi and other organisms which may bring about rotting of the seeds. Depending on the type of seed, the optimal moisture content is between 5 and 15 per cent.

Steps to Moisture Content Determination:

The following steps can be used to test the moisture content of seeds:

1. Prepare some seeds as a sample. The sample size needs to be sufficient to serve as an accurate representation of the complete batch of seeds.
2. Weigh the seed sample.
3. The sample of seeds is dried in an oven at a particular temperature for a particular amount of time.
4. Weigh the dried seed sample.
5. Make a moisture content calculation. To do this, multiply the result of dividing the weight of the original sample by the weight of the lost water by 100 per cent. The formula to calculate Mc is given below:

$$\text{Moisture content (\%)} = \frac{(\text{Weight of seeds} - \text{Weight of dry seeds})}{\text{Weight of dry seeds}} \times 100$$

Testing the moisture content of seeds can be done using a variety of techniques. Broadly, these methods may be classified into two groups:

1. Direct Methods
2. Indirect methods

1. Direct Methods:

This approach is straightforward and precise and is mostly used for research, however, it takes more time than indirect approaches. Examples of direct approaches are the Brown-Duvel distillation method and the hot air oven method.

If the sample's moisture content is less than 13 percent, the hot air oven procedure can be completed in a single step, or if the sample's moisture content is greater than 13 percent, it can be completed in two steps.

a. Single stage method:

1. Grind the grains into a 2-3 gram powder.
2. Maintain the ground sample in a hot air oven at 130°C for 1 hour.
3. Take out the sample, put it in a desiccator, and then weigh it after an hour.
4. Use the aforementioned procedure (formula) to determine the moisture content.

b. Double stage method:

1. Place a 25–30 gram sample of whole grains in a hot air oven set at 130°C for 14–16 hours to reduce the moisture content to roughly 13 percent.
2. Carry out the single-stage method's instructions.

2. Indirect methods:

The foundation of indirect methods is the measurement of some grain parameters that are reliant on the grain's moisture content. Electrical resistance and dielectric techniques are two instances of indirect approaches.

The air-oven approach is the most popular. Although this approach is quite easy to follow and straightforward, it can take some time.

Some Other methods:

Other methods of testing moisture content include:

1. Vacuum drying method
2. Microwave drying method
3. Near-infrared spectroscopy (NIRS)

The NIRS technique is the most expensive and most accurate way to measure moisture content.

An essential component of seed quality control is the testing of seeds for moisture content. It aids in ensuring that seeds are of a high quality and will germinate successfully. The environment, farmers, and the world's food supply all benefit from this.

Advantages of Seed Moisture Content Determination:

Advantages of seed testing for moisture content are hereunder:

1. It guarantees that seeds are of a high quality and will germinate successfully.
2. It protects farmers against purchasing seeds of inferior or dubious quality.
3. It provides protection against the spread of diseases/pathogens and insect-pests in the environment/region.
4. It encourages fair practices in the trading of seeds.
5. Crop productivity and food security are improved.

(D) TESTINGSEEDS FORDISEASE(S):

An essential component of seed quality control is testing seeds for the presence of disease(s). It aids in ensuring that seeds are of a high quality and will germinate successfully. The environment, farmers, and the world's food supply all benefit from this.In order to find out the presence of diseases or disease-causing agents/pathogens in seeds, disease testing is done. This is accomplished by looking for disease indicators on the seeds, such as mould or rot.

By identifying the presence of disease-causing pathogens in a sample, seed disease testing is a technique for assessing the quality of seeds. In order to make sure that seeds are of excellent quality and will produce good level of germination, it is a crucial component of seed quality management.

Pathogens like as fungi, bacteria, viruses, and nematodes can all contribute to seed infections and lead to deleterious effects of seed performance. These diseases can harm seedlings, inhibit seed germination, and lower agricultural yields.

Steps to testing seeds for disease(s):

These steps can be used to test seeds for diseases:

1. Prepare some seeds as a sample. The sample size needs to be sufficient to serve as an accurate representation of the complete batch of seeds.
2. Check the seeds for disease symptoms including mould, rot, or discolouration lumpiness and other visible symptoms/signs.
3. Gather seed samples, then send them to the lab for additional analysis.
4. The laboratory will examine the seeds for the presence of pathogens and infections in a number of ways, including:

a. Visual assessment and examination
b. Microscopy
c. Culture
d. Molecular analysis

The outcomes of the seed testing will show whether or not there are any illnesses in the sample. If diseases are discovered, the laboratory will describe the disease and its level of severity for the lot being proceeded as seed.

Advantages of testing seeds for disease(s):

Following are some of the advantages of seed testing for diseases:

1. It guarantees that seeds are of a high quality and will germinate successfully to he level indicated.
2. It protects farmers against purchasing seeds of dubious or inferior quality.
3. It protects against the spread of disease in the environment/region.
4. It encourages fair practices in seed trading.
5. Crop productivity and food security are improved.

The regulation of seed quality includes seed testing. It aids in guaranteeing that seeds are of a high quality and will produce good germination. For farmers, the environment, and the world's food supply, this is significant.

A number of other tests can be used to assess seed quality in addition to the methods already described. Tests for Seedling vigour, seedling emergence, and seedling growth are among them. The genetic purity of seeds can also be determined by seed testing.

The procedure of testing seeds is intricate and crucial. To guarantee that seeds are of high quality and will produce desired level of germination, it is crucial. A useful tool for growers, seed suppliers, and regulatory organizations is seed testing. It promotes fair trade, increases crop yields, and increases crop security and environmental protection.

Organizations offering seed testing services:

Here are some of the organizations that offer seed testing services:

1. American Seed Trade Association (ASTA)
2. Association of Official Seed Analysts (AOSCA)
3. International Seed Testing Association (ISTA)
4. National Seed Storage Laboratory (NSSL)
5. State seed testing laboratories

You can get more information by contacting one of these organizations if you are interested in getting your seeds tested.

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Seed Heath Testing: A Brief Outline

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A healthy seed of a high yielding variety is considered as one of the most important inputs for raising a healthy crop to meet the ultimate goal of food security worldwide. Further, contamination of a seed or a seed lot with pathogen structures serves as source of primary inoculum which is directly related with disease severity. Hence, it is of paramount important to detect the presence of pathogen inoculum in or on seed or within seed lot as a separate contaminant is of high value. There are various indigenous and advanced techniques including molecular techniques for seed testing. The seed borne inoculum present as externally and internally as well as, separate contaminants should be eliminated by using various physical and non-chemical methods for ecological sound management thereof. Further, seed borne diseases can be managed by legislative measures by adapting domestic and international quarantine including diseases caused by plant pathogenic nematodes.

There are three primary organizations that publish standardized seed health test methods for use in international trade. They are International Seed Testing Association (ISTA), International Seed Health Initiative(ISHI), and in the United States, the National Seed Health System (NSHS) (Munk v old, 2009). Two of the most important concepts in seed health testing are sensitivity and selectivity, which are inextricably linked, e.g., increasing the selectivity of semi selective media may decrease the recovery efficiency of all or some strains of the target organism. On the contrary, increasing selectivity may decrease the number of non-target organisms that act as competitors and/or inhibitors that obstruct with the assay, and thus increase the detection sensitivity (Roumagnace *et al.,* 2000; Toussaint et al., 2001; Wydra *et al.,* 2004). A semi-selective medium may have a higher mean plating efficiency than a standard growth medium because standard media are complex and often become toxic, perhaps due to the accumulation of peroxides or other secondary metabolites (Block et al., 1998;Pataky et al., 1995).Infection rate of seeds depending on some environmental conditions such as high relative humidity, suitable temperature and also high level of moisture content in seed is variable. The study of seed-borne pathogens is necessary to determine seed health and to improve germination potential of seed which finally leads to increase of the crop production. Seed health testing to detect seed-borne pathogens is an important step in the management of crop diseases (Haji Hasani*et al.,* 2012; Tsedaley 2015).

Why seed health testing is required?

Seed Heath Testing is necessary as seed-borne inoculum give rise to disease development. Further, imported seed lots may introduce diseases in new areas. It is also important to elucidate the causes of poor germination or field establishment. Seed Health Testing also indicate the necessity to carry out seed lot treatment(s) to eradicate seed-borne pathogens.Seed borne pathogens may be fungi, bacteria and viruses. Seed borne pathogens may be of two types, viz., externally seed born and internally seed born. Seed borne diseases assumed a greater importance to seed industries. Seedborne pathogens results in seed rots, seedling decay, pre and post emergence mortalities, abnormalities, discoloration,

reduced seed size and shriveling of seeds. The seed borne pathogens not only affect the market value but also nutritive value of the products.

Losses due to seed borne diseases can be summarized as follows:

Seed borne diseases caused severe loss with respect to loss of human life and Income. Late blight of potato caused by *Phytophthorainfestans*was responsible for A healthy seed of a high yielding variety is considered as one of the most important inputs for raising a healthy crop to meet the ultimate goal of food security worldwide. Further, contamination of a seed or a seed lot with pathogen structures serves as source of primary inoculum which is directly related with disease severity. Hence, it is of paramount important to detect the presence of pathogen inoculum in or on seed or within seed lot as a separate contaminant is of high value. There are various indigenous and advanced techniques including molecular techniques for seed testing. The seed borne inoculum present as externally and internally as well as, separate contaminants should be eliminated by using various physical and non-chemical methods for ecological sound management thereof. Further, seed borne diseases can be managed by legislative measures by adapting domestic and international quarantine including diseases caused by plant pathogenic nematodes.

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Table 1: Losses due to seed borne diseases in some crops

Crop	Disease/ Pathogen	Yield loss (%)
Rice	Blast	75
	Bacterial Blight	6-60
	Brown spot	50-90
	Sheath Rot	50
Wheat	Loose smut	100
	Ear cockle	100
Chickpea	Ascochyta blight	80-90
Cowpea	Anthracnose	30-35
Soya bean	Tobacco bud blight	66
	Soya bean mosaic virus	25-94
Cucumber	Cucumber mosaic virus (planting of 0.5% infected seeds)	34-53

Table 2: Important seed borne diseases

Crop	Disease	Causal organism
Brinjal	Fruit rot	*Phomopsis vexans*
Carrot	Black root rot or Seedling blight	*Alternaria radicina* *A. dauci*
Onion	Damping off Downy mildew Purple blotch Stemphylium Blight	*Botrytis allii* *Peronospora destructor* *Alternaria porri* *Stemphylium vesicarium*
Pepper and chilli	Anthracnose or fruit rot	*Colletotrichum capsici*
Radish	Grey leaf spot Leaf spot	*Alternaria brassicae* *A. raphani*
Crucifers	Grey and black leaf Spot	*Alternaria brassicae* *A. brassicicola*
Tomato	Buck eye rot Damping off Early Blight Late blight or Fruit rot	*Phytophthoraparasitica* *Pythiumaphanidermatum* *Alternaria solani* *Phytophthora infestans*
Wheat	Loost smut Karnal smut Flag smut	*Ustilagosegetum var. tritci* *Neovossia indica* *Urocystis agropyri*
Rice	Bunt False Smut Stack burn	*Neovossia horrid* *Ustilaginoidea virens* *Pyricularia oryzae* *Trichoconiella padwickii*
Maize	Black kernel rot Cob rot Southern leaf blight	*Botryodiplodia theobromae* *Fusarium moniliformae* *Drechslera maydis*
Pearl millet	Downy mildew Smut	*Sclerospora graminicola* *Tolyposporium penicillariae*
Sorghum	Anthracnose Kernel or grain smut Downy mildew	*Colletotrichum graminicola* *Sphacelotheca sorghi* *Peronosclerospora sorghi*
Cotton	Anthracnose Wilt Alternaria blight	*Colletotrichumindicum* *F.oxysporumf.sp.vasinfectum* *Alternaria macrospora*
Sugarcane	Red rot Wilt Pineapple Disease Smut Mosaic(s)	*Colletotrichumfalcatum* *Fusarium moniliforme* *Ceratocystisparadoxa* *Ustilago scitaminea* *Virus*
Groundnut	Charcoal rot Crown rot Yellow mould/Aflatoxin	*Macrophomina phaseolina* *Aspergillus niger* *Aspergillus flavus*
Sunflower	Alternaria blight Downy mildew Charcoal rot	*Alternaria helianthi* *Plasmopara halstedii* *Macrophomina phaseolina*
Soybean	Anthracnose Pod & stem blight	*Colletotrichum dematium* *Phomopsis sojae*

	Purple seed stain	*Cercospora kikuchii*
Chickpea	Ascochyta blight Wilt Gray mold Alternaria blight	*Ascochyta rabiei* *Fusarium oxysporum* f.sp. *ciceri* *Botrytis cinerea* *Alternaria circinum*

Table 3: Seed borne viral diseases

Virus name	Crop
Tobacco mosaic virus	Tomato
Alfa-alfa mosaic virus	Alfa-alfa
Crinkle virus	Black gram
Cucumber mosaic virus	Cucumber
Sugarcane grassy stunt virus	Sugarcane
Citrus ring spot virus	Citrus
Banana bunchy top virus	Banana
Cowpea mosaic virus	cowpea

Seed Health Testing

Seed health is a measure of freedom of seeds from pathogens. The presence or absence of seed-borne pathogens can be confirmed through the use of seed health testing (Agrawal, 1995). The term "seed health" includes the incidence in the seed lot of fungi, bacteria, viruses, and animal pests such as nematodes and insects. The test used depends on the organism being tested for and the purpose of the test quality assurance or phyto-sanitary purposes when seed is exported (ISTA, 2009b). It includes visual examination of seeds externally or internally, macro or microscopically for the presence of pathogens as well as incubating seeds on agar or moist blotter papers and identifying the pathogens microscopically (Warham*et al*., 1990). Many detection assays exist for different seed borne pathogens; however, few satisfy the minimum requirements for adequate seed tests. Ideally, seed assays should be sensitive, specific, rapid, robust, inexpensive and simple to implement and interpret (Walcott, 2003).

Seed testing is necessary for a number of reasons: to determine the quality of the seed based on a number of seed quality attributes; to provide a basis for price and consumer discrimination among seed lots and seed sources; to determine the source of a seed problem, thereby facilitating any corrective measure(s) that maybe required; and to fulfil legal and regulatory requirements for certified seed classes and allow for seed movement across international boundaries (FAO, 2010). There are six main requirements for selection of seed health tests methods (Amare, 2007/8). These are:

-Specificity: the ability to distinguish the target pathogen from all organisms likely to occur on seeds from field or store, i.e., to avoid false positives.

-Sensitivity: the ability to detect target organisms, which are potentially significant in field crops at a low incidence in seed stocks.

-Speed: in some cases, small concession to accuracy may be necessary to ensure rapid results, but such results should be followed by more definite testing.

- Simplicity: the methodology should minimize the number of stages to reduce room for error and to enable tests to be performed by not necessarily highly qualified staff.
- Cost effectiveness: test costs should form part of acceptable production margins for each crop.
- Reliability**:** test methods must be sufficiently robust so that results are repeatable within and between samples of the same stock regardless of who performs the test (within the bounds of statistical probability and sample variation).

Alerts issued against seed borne diseases

Alerts are issued based on severity of the diseases caused by a seed borne pathogen. There are three types of alerts issued based on severity of the diseases. Red Alert is issued against those diseases which are highly virulent, and highly seed-borne in nature. Similarly, Orange Alert is issued against those diseases which are moderately virulent and highly seed-borne or highly virulent and moderately seed-borne in nature. The Yellow Alert diseases are those which are Moderately virulent and seed-borne in nature. The examples of Red Alert diseases are *Brassica* Black Rot Bacteria (*Xanthomonas campestris*); Brassica Blackleg Fungus (*Phoma lingam / Leptosphaeria maculans*); *Lettuce Mosaic Virus*; Carrot Bacterial Blight (*Xanthomonas campestris* pv. *carotae)*; *Tomato Mosaic Virus*; etc. Similarly, theorange alert diseases includeCarrot Fungal Blights caused by Alternaria blight (*Alternaria dauci*)*,* Cercospora blight (*Cercospora carotae*); Tomato bacterial diseases caused by Bacterial canker (*Corynebacterium michiganense* pv. *michiganense*), Bacterial Spot (*Xanthomonas campesiris* pv. *vesicatoria*), Bacterial Speck (*Pseudomonas syringae*); Onion white rot fungus (*Sclerotinia sclerotiorum*).

Historically seed health tests have been classified into the following four distinct groups based on the general techniques used to observe the target pathogen such as Direct Inspection, Incubation Tests, Examination of the embryo (embryo count method), Immunoassays and Molecular Methods.

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Sugarcane	Red rot Wilt Pineapple Disease Smut Mosaic(s)	*Colletotrichumfalcatum* *Fusarium moniliforme* *Ceratocystisparadoxa* *Ustilago scitaminea* *Virus*
Groundnut	Charcoal rot Crown rot Yellow mould/Aflatoxin	*Macrophomina phaseolina* *Aspergillus niger* *Aspergillus flavus*
Sunflower	Alternaria blight Downy mildew Charcoal rot	*Alternaria helianthi* *Plasmopara halstedii* *Macrophomina phaseolina*
Soybean	Anthracnose Pod & stem blight Purple seed stain	*Colletotrichum dematium* *Phomopsis sojae* *Cercospora kikuchii*
Chickpea	Ascochyta blight Wilt Gray mold Alternaria blight	*Ascochyta rabiei* *Fusarium oxysporum* f.sp. *ciceri* *Botrytis cinerea* *Alternaria circinum*

Table 3: Seed borne viral diseases

Virus name	Crop
Tobacco mosaic virus	Tomato
Alfa-alfa mosaic virus	Alfa-alfa
Crinkle virus	Black gram
Cucumber mosaic virus	Cucumber
Sugarcane grassy stunt virus	Sugarcane
Citrus ring spot virus	Citrus
Banana bunchy top virus	Banana
Cowpea mosaic virus	cowpea

Seed Health Testing

Seed health is a measure of freedom of seeds from pathogens. The presence or absence of seed-borne pathogens can be confirmed through the use of seed health testing (Agrawal, 1995). The term "seed health" includes the incidence in the seed lot of fungi, bacteria, viruses, and animal pests such as nematodes and insects. The test used depends on the organism being tested for and the purpose of the test quality assurance or phyto-sanitary purposes when seed is exported (ISTA, 2009b). It includes visual examination of seeds externally or internally,macro or microscopically for the presence of pathogens as well as incubating seeds on agar or moist blotter papers and identifying the pathogens microscopically (Warham*et al*., 1990). Many detection assays exist for different seed borne pathogens; however, few satisfy the minimum requirements for adequate seed tests. Ideally, seed assays should be sensitive, specific, rapid, robust, inexpensive and simple to implement and interpret (Walcott, 2003).

Seed testing is necessary for a number of reasons: to determine the quality of the seed based on a number of seed quality attributes; to provide a basis for price and consumer discrimination among seed lots and seed sources; to determine the source of a seed problem, thereby facilitating any corrective measure(s) that maybe required; and to fulfil legal and regulatory requirements for certified seed classes and allow for seed movement across international boundaries (FAO, 2010). There are six main requirements for selection of seed health tests methods (Amare, 2007/8). These are:

- Specificity: the ability to distinguish the target pathogen from all organisms likely to occur on seeds from field or store, i.e., to avoid false positives.
- Sensitivity: the ability to detect target organisms, which are potentially significant in field crops at a low incidence in seed stocks.
- Speed: in some cases, small concession to accuracy may be necessary to ensure rapid results, but such results should be followed by more definite testing.
- Simplicity: the methodology should minimize the number of stages to reduce room for error and to enable tests to be performed by not necessarily highly qualified staff.
- Cost effectiveness: test costs should form part of acceptable production margins for each crop.
- Reliability**:** test methods must be sufficiently robust so that results are repeatable within and between samples of the same stock regardless of who performs the test (within the bounds of statistical probability and sample variation).

Alerts issued against seed borne diseases

Alerts are issued based on severity of the diseases caused by a seed borne pathogen. There are three types of alerts issued based on severity of the diseases. Red Alert is issued against those diseases which are highly virulent, and highly seed-borne in nature. Similarly, Orange Alert is issued against those diseases which are moderately virulent and highly seed-borne or highly virulent and moderately seed-borne in nature. The Yellow Alert diseases are those which are Moderately virulent and seed-borne in nature. The examples of Red Alert diseases are *Brassica* Black Rot Bacteria (*Xanthomonas campestris*); Brassica Blackleg Fungus (*Phoma lingam / Leptosphaeria maculans*); *Lettuce Mosaic Virus*; Carrot Bacterial Blight (*Xanthomonas campestris* pv. *carotae)*; *Tomato Mosaic Virus*; etc. Similarly, theorange alert diseases includeCarrot Fungal Blights caused by Alternaria blight (*Alternaria dauci*)*,* Cercospora blight (*Cercospora carotae*); Tomato bacterial diseases caused by Bacterial canker (*Corynebacterium michiganense* pv. *michiganense*), Bacterial Spot (*Xanthomonas campesiris* pv. *vesicatoria*), Bacterial Speck (*Pseudomonas syringae*); Onion white rot fungus (*Sclerotinia sclerotiorum*).

Historically seed health tests have been classified into the following four distinct groups based on the general techniques used to observe the target pathogen such as Direct Inspection, Incubation Tests, Examination of the embryo (embryo count method), Immunoassays and Molecular Methods.

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Seed Testing: An Overview

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Seed is a vehicle for delivery of improved technologies and is a mirror for portrayal of inherent genetic potential of a variety/ hybrid. Seed offers to integrate production, protection and quality enhancement technologies in a single entity, in a cost effective way. Seed can play pivotal role in achieving higher productivity, use of quality seeds alone could increase productivity by 15-20 % highlights the important role of seed in agriculture. Seed is the critical input for achieving sustainable production and efficacy of all other inputs depends upon quality supply of seed to farmers at right time.

India is predominantly agrarian country and agriculture contributes around 14 % of gross domestic product (GDP). Agriculture as a livelihood, directly supports 59 % of Indian work force thus, any technological intervention will have multifold effect on millions of poor people engaged in agriculture and allied activities. Over the last few decades, national seed research system able to overcome many challenges in field of seed science and technology resulting in supply of improved seeds to end-users. However, to ensure every seed produced to be of utmost quality there is need to streamline seed research system to deliver commercially viable technologies in seed production, seed storage and processing, seed quality enhancement and seed quality control etc.

Seed quality assurance is a mechanism put in place to guarantee the quality of seed from production, harvesting, and post-harvest handling through sales. Seed quality assurance is a systematic and planned procedure for ensuring the genetic, physical and physiological integrity of the seed delivered to farmers. The development of high yielding varieties with good seed testing will ensure delivery of quality seed to the stakeholders. The testing methods will play an important role in ensuring the quality of seeds. In the last two decades there were numerous advances and updating of existing seed testing methods. In this age of precision seed and custom seed testing, the updating of the procedures and development of new one is very important. In this context, an exhaustive review of the new and updated seed testing procedures have been made hereunder.

Seed testing

Seed testing is determining the standards of a seed lot viz., physical purity, moisture, germination and ODV and thereby enabling the farming community to get quality seeds. The Seed Testing Laboratory is the hub of seed quality control. Seed testing services are required from time to time to gain information regarding planting value of seed lots. Seed testing is possible for all those who produce, sell and use seeds.

Objective of Seed Testing

Seed testing is required to achieve the following objectives for minimising the risks of planting low quality seeds.

1. To identify the quality problem and their probable cause

2. To determine their quality, that is, their suitability for planting
3. To determine the need for drying and processing and specific procedures that should be used
4. To determine if seed meets established quality standards or labelling specifications.
5. To establish quality and provide a basis for price and consumer discrimination among lots in the market. The primary aim of the seed testing is to obtain accurate and reproducible results regarding the quality status of the seed samples submitted to the Seed Testing Laboratories.

Importance of seed testing

- The importance of seed testing was realized more than 100 years ago for assured planting values. The adulteration of vegetable seeds by stone dust which was packed in some parts of the world particularly in Europe.
- Seed testing has been developed to aid agriculture to avoid some of the hazards of crop production by furnishing the needed information about different quality attributes *viz.,* purity, moisture, germination, vigour and health.
- Quality control of seed depends on the different seed testing protocols which determine the genuineness of the cultivar.
- Testing of seed to evaluate the planting value and the authenticity of the certified lot.
- Seed testing is required to assess the seed quality attributes of the seed lots which have to be offered for sale.
- These quality attributes are seed moisture content, germination and vigour, physical and genetic purity, freedom from seed borne diseases and insect infestation. In India, seed testing is done mainly for moisture, germination and physical purity of seeds.
- Standard seed testing procedures for the evaluation of the seeds were developed by ISTA. It is obligatory on the part of the seed analyst to follow rules prescribed by 1STA (1STA, 1985) if the seed is moving to the International trade.
- The seed testing procedures which are described below are based mostly on the international rules because most of our rules are based on, 1STA, 1996. Economic yield of a crop depends on the quality of seeds which can be evaluated by seed testing (1STA, 1996).
- The testing of seed quality is carried out on seed samples drawn from seed lot to be used for cultivation. The quantity of seed sample taken for testing in laboratory is minute compared to that of seed lot it represents.

Innovative methods of Seed Testing:

Seed Sampling

Pneumatic seed samplers: It can obtain seed samples from deep silo bins. Preferred locations are the centre core and near walls warmed by the sun or other heat sources. Sections of sampling tube are attached to a cyclone air pump, which provides the suction force for pulling up the sample grain and for pushing the probe further into the mass. Using this equipment, two people can make six or seven 24-m probes during a working day, but this may vary with the type of grain and its moisture content.

Automatic Samplers: It is a device mounted on the seed stream. Primary samples are drawn from the seed stream automatically usually by time intervals. Automated samplers are those placed in a conditioning line to take samples from time to time while the seeds is flowing sampling intervals, as well as the quantity of seed taken with each sample, are usually adjustable.

Physical Purity Test

ERGO vision system for purity testing:

Operation of ERGO vision system:

- Working sample is placed in the sample holding funnel, the seed flow the funnel to the trays that are calibrated to desired level
- The tray moves the seeds to the inspection tray where seeds are inspected the speed of the seed flow can be adjusted
- The seeds are examined using a high quality microscope, magnification can be adjusted
- The flow of the seeds can be stopped at any point time to make closer examination
- Inspected seeds are automatically deposited from the inspection tray in to the sample holding cup.

Multispectral imaging analysis: Determination of purity of seed lots in seed testing is often time consuming, and for some species difficult. This is especially the case for seed lots in which the seeds of different subspecies can hardly be distinguished visually. Multispectral imaging analysis may provide tools for distinguishing species and varieties.

Images from each seed sample are captured using a Videometer Lab instrument. This instrument acquires multispectral images in 19 wavelengths (375, 405, 435, 450, 470, 505, 525, 570, 590, 630, 645, 660, 700, 780, 850, 870, 890, 940 and 970 nm). The Sensors 2015, 15 4499 instrument consists of a sphere, which is coated with matte titanium paint, and it ensures that light is scattered evenly around the object. The 19 light emitting diodes (LED) are placed along the rim of the sphere and a camera is top-mounted. Before images are captured the instrument is calibrated in respect to color, geometry and self-illumination to ensure a set of direct comparable images. After capturing the images data extraction and transformation of pixel data are done in the Videometer Lab software (version 2.13.83). Different algorithms are used for the data analysis.

Seed Moisture Estimation

Digital seed moisture meters:

✓ Digital seed moisture meter with digital display, the integration of the human nature design and high sensitivity of the sensor can according to the variation of grain moisture and output the corresponding changes of electrical signals, the measured results visually displayed on the screen.

✓ Measuring speed can replace traditional oven method, make water shorten test time, the whole process of operation for only 1 minute, measured value 1 seconds reading, greatly save the inspectors' valuable time.

✓ Small volume, light weight, strong anti-jamming ability, can carry on site for rapid detection

✓ The temperature and humidity compensation technology, high measurement precision, accurate and reliable, stability is good.

✓ Instrument design USES the large scale integrated circuit with low consumption and liquid crystal display technology, low power consumption.

Seed Viability and Germination Test

Spectral imaging analysis

Speed, accuracy and costs are important aspects of seed testing methods. In this respect, spectral imaging technology can be seen as a methodology which can add to the knowledge of seed quality aspects, the speed of testing and the reproducibility of traditional tests within and between laboratories. Seed size, shape and colour are common features that are employed as sorting parameters for improvement of seed quality. Features can be extracted from images captured at different wavelengths. These features provide a new opportunity of determining seed quality parameters such as morphological and biochemical characteristics of the seed coat, for example the presence of waxes, pectin and phenols.

Images of an object exposed to light of different wavelengths can be obtained by digital cameras. In general, digital cameras capture three different images: a red, a green and a blue (RGB) colour image, by using three different sensors (for red, green and blue light). The combination of these images produces a colour picture. When images of for example green seeds are taken by such a camera, the sensors will detect the green colour because green light waves are reflected by the seeds, whereas blue and red light waves are absorbed. In spectral imaging the sequential exposure of the object to light of different wavelengths provides further information about topographical texture, spectral texture and gloss. An example of this is the possibility to distinguish between the presence of chlorophyll a and b by the use of specific wavelengths in the region of 400– 500 nm and 600–700 nm, which would not be detected in the traditional colour image (RGB). A spectral image primarily provides information on properties originating in the surface chemistry and structure. In multispectral imaging, several images are taken in selected bands of wavelengths; each represents an individual wavelength, depending on the selected light sources and optical filters. Hyperspectral imaging deals with several images that cover a complete spectral range with a specific interval between each wavelength.

Current multispectral imaging systems will typically use light emitting diodes (LEDs) as light sources with wavelength bands of 10–25 nm width (FWHM), whereas hyperspectral systems use broadband light sources covering the full spectral range and

then separate the wavelength on the camera side, e.g. using a diffraction grating. By using multispectral imaging it is possible to apply only those bands which are valuable in discriminating between the required traits of the seed. Advances resulting from the use of a system in which selected frequencies of wavelengths are employed (e.g. by LED) are opportunities to determine the presence of specific biochemical compounds in or on the surface of the seed, fungi or seedling. These may be various pigments, wax, chlorophyll or fatty acids. Furthermore, taking of images by use of near-infrared (NIR) wavelengths provides opportunities to separate features that cannot be done visually (Singh *et al.,* 2007), like lipids, proteins and carbohydrates.

The use of image analysis technology which can mimic human vision will eliminate subjective assessment, and also provide documentation. When dealing with multi-dimensional data, as generated in multispectral images, reduction of the data dimensions is required to visualize and find natural groupings within the data set. Data reduction can be achieved by various mathematical methods which transform multispectral images into new images with reduced numbers of bands. If the various groups within the samples are unknown, it can be valuable to apply unsupervised data analysis methods, such as principal component analysis (PCA). Examples of the possibilities of spectral imaging in seed quality testing are described below.

Purity

Multispectral imaging analysis may provide tools for distinguishing species and varieties. In maize and grape, spectral imaging has been used to distinguish varieties based on the optical spectral features of the seeds (Zhang *et al.,* 2012; Rodríguez- Pulido *et al.,* 2013). For maize, the optimal wavelengths for distinguishing the groups (i.e. varieties) in a principal component analysis (PCA) were 523, 579 and 863 nm. Measurements on these three wavelengths allowed the varieties in the batch to be clearly distinguished.

Germination and vigour

Monitoring of seed germination by imaging has been employed in a number of studies. Multispectral imaging may contribute to identifying morphological and biochemical features of the seed or radical that can provide valuable information relating to germination and seed vigour. Image analysis has proven to be a valuable tool in germination testing and monitoring of germination curves, as discussed by Matthews and Powell (2012), Dell'Aquila (2004, 2005) and Wagner *et al.* (2012), and determination of pregerminated barley (Arngren *et al.,* 2011).

Seed health

In a multispectral imaging approach, the combinations of the features from images captured by visible light wavelengths and NIR wavelengths were shown to be valuable in separating uninfected spinach seed from seeds infected by *Stemphylium botryosum*, *Cladosporium* spp., *Fusarium* spp., *Verticillium* spp. or *Alternaria alternate* (Olesen *et al.,* 2011).

X-ray Method:

The X-ray test is a quick, non-destructive method to evaluate seed quality. The X-ray images of seeds provide information not only about their morphology and development, but also on the deformations of the embryo, the micro fractures, the endosperm filled and its density, the mechanical, physical, and insect damages. That consists of the visualization of the internal seed structure on an X-radiograph. An X-ray gives a clear image of the internal morphology of the seed or fruit. The X-rays are generated using very low voltages (much lower than are used for most medical applications) and the method is thus safe with the appropriate precautions. Visual checking of seeds can give us an indication that they may not germinate. Visual examination of a seed cannot tell us that a seed is capable of germination, it can give us an indication that the seed will not germinate.

X-ray radiography has been used since the fifties for determining several seed quality aspects. Looking at the seed interior, it can be assessed whether all the essential seed components are adequately present. Upgrading, based upon X-ray images, therefore appears to be a very promising method, with little waste compared to conventional upgrading techniques. Indeed, several investigations have shown that by using X-ray radiography and manual selection, germination characteristics can be improved.

Working Principle: X-rays are a form of electromagnetic energy. They travel at the same velocity as light. They can penetrate different material that absorbs. "The principle of X-ray is differentially absorbed by the different parts of the seeds, depending up on their thickness, which can be recorded on a film. X-ray that pass through and strike a photographic material (film) form the image called radiograph.

Seedling Vigour Test

Chlorophyll fluorescence Test (CFT)

Chlorophyll is often present in seeds during their early development. A relationship between chlorophyll content and seed quality was discovered as early as 1989 (Steckel *et al.,* 1989). During maturation, the chlorophyll content of a seed generally decreases gradually. Consequently it is related to the maturity of the seed (Smolikova *et al.,* 2011). The chlorophyll level can be assessed by destructive chemical extraction methods followed by optical detection (Breia *et al.,* 2013, Onyilagha *et al.,* 2011), but this is less convenient for routine testing. A new method has been developed, based on the detection of chlorophyll by its fluorescence. This new method can determine the seed chlorophyll content quickly and non-destructively.

Working principle: The principle of CF is that light of a certain wavelength is emitted by a suitable source (LED, laser) and shone onto a seed. As a result of the absorption of this light, the chlorophyll in the seed emits fluorescent light of a slightly longer wavelength. This emitted fluorescent light is captured by a camera or photo multiplier and thereby transformed into a small current of a few picoampères (pA). The strength of the current is related to the intensity of the fluorescence, and hence to the chlorophyll content of the seed. The chlorophyll content in seeds is related to maturity in many species, and thus to seed quality parameters. In general, chlorophyll declines with increasing seed maturity and

quality. The higher level of chlorophyll indicates seeds had poorer seedling vigour and field performance.

Real-time Q_2 measurements: This technology measures the oxygen consumption of single seeds in a closed environment. The total test is therefore performed under increasing stress conditions (oxygen stress) and gives us a deeper insight in various aspects of the seed quality. Q_2 analysis technology brought a revolutionary breakthrough to seed quality testing, allowing the method of measuring seed germination level faster and more accurately. For different crops, the expected measure time ranges from 10 hours to 48 hours. We can easily determine whether the seed is in dormancy or active. Q_2 analysis technology can rapidly and exactly demonstrate vigour and uniformity degree of the seeds. This technology uses wells of ELISA plates in which seeds are placed individually. The cells are covered with a specially coated foil, which if excited with a laser, produces fluorescence this fluorescence is then a measure of the oxygen content of the cell. The rate of oxygen consumption is a measure of quality, which we would call differences in vigour.

Genetic Purity Test

Determination of seed genetic purity by conventional (GOT) and Non-conventional methods like advanced tools by using molecular markers, proteomics etc.

Molecular marker: Unique sequence of nucleotides found on a strand of DNA. Sax (1923) first reported association of a simply inherited genetic marker with a quantitative trait in bean.

Different Markers that are being used in assessment of genetic purity of seed lots

RAPD: DNA markers which developed by amplifying random sequence of specific markers through the use of random primers
SSR: A particular target sequence amplified using forward and reverse primer
SNP: DNA markers in which their polymorphism can be determined by single nucleotide difference
RFLP: It is a variations found within a species in the length of DNA fragment generated by specific endonucleases
AFLP: These are differences in restriction fragment length caused by SNPs or INDELs that create or abolish REs recognition sites
CAPS: It is polymorphism that are found in restriction fragment length caused by SNPs or INDELs that create or abolish REs recognition sites in PCR amplicons produced by locus specific oligonucleotide primers
EST-SSR: Expressed sequence Tags are short sequences from one or other end of the expressed gene
ISSR: A variant of polymerase chain reaction that uses SSR primers to amplify the regions between their target sequences.

Advantages of molecular markers in seed testing:

- ✓ Replacement of traditional method of seed testing

- ✓ Accurate evaluation of Seed quality
- ✓ Quick and Rapid method of evaluation of seed quality
- ✓ Characterization of variety become easy
- ✓ Less Space and Laborious over traditional methods
- ✓ Results are reproducible
- ✓ Not affected by environment
- ✓ Large number of samples can test at a time

Seed Health Testing

NASH (Nucleic Acid Sequence or Spot Hybridization) technique:

NASH is a new technology relies on the unique ability of nucleic acid molecules to hybridize specifically with molecules having complementary sequences. This technique was developed by E M Southern (1975). It's based on the principle that hybridization (binding) of complementary DNA sequences. This relatively new technology relies on the unique ability of nucleic acid molecules to hybridize specifically with molecules with complementary sequences

Types:

1. Southern hybridization - hybridization of a probe to filter bound DNA
2. Northern hybridization **-** hybridization of a probe to filter bound RNA

Materials required:

- ✓ DNA probe - a single-stranded DNA sequences, used to identify a complimentary nucleic acid sequence.
- ✓ Nucleic acid- infected seed
- ✓ Restriction enzymes
- ✓ EtBr - fluorescent dye which intercalates into the DNA molecule, enables visualization of DNA under UV light
- ✓ Nylon or nitrocellulose membrane – flat form for fixation nucleic acid
- ✓ Agarose gel & Buffer etc.

Enzyme-linked Immuno Sorbent Assay (ELISA):

ELISA is the most widely used serological test that relies on interaction between antibodies generated against unique antigens on the surface of the plant pathogens. Serological seed assays rely on antibodies (polyclonal or monoclonal) generated against unique antigens on the surfaces of plant pathogens. Antibodies bind strongly and specifically to their antigens and can subsequently be detected by the enzymatic digestion of substrates or fluorescent tags. Serological assays do not require pure isolations of the pathogen and, hence, are applicable to biotrophic and necrotrophic seedborne pathogens. Currently serology is the most widely used detection assay for seedborne viruses and it has proven to be sensitive and robust. Serology has also been widely used for the detection of bacterial and fungal plant pathogens, but the unavailability of species-specific antibodies is

a limitation. Additionally, the detection thresholds of serology-based assays vary significantly based on the quality of the antibody and the testing format.

Polymerase Chain Reaction (PCR):

Polymerase chain reaction (PCR) is the in-vitro, primer-directed, enzymatic amplification of nucleic acids. This technique has been used in many diverse applications including diagnosis of plant diseases. For PCR, primers (small oligonucleotide probes) designed to anneal to specific DNA sequences in the target organism's chromosomal DNA or RNA, hybridize with and direct amplification of millions of copies of the target sequence. This amplified DNA can be visualized after electrophoresis in ethidium bromide stained agarose gels. PCR has many beneficial characteristics that make it highly applicable for detecting seed borne pathogens. These include speed (completed within 2 to 3 h); specificity (DNA probes can be designed to amplify nucleic acids from the desired genus, species, subspecies, race, etc.); sensitivity (single copies of nucleic acids can be detected after amplification) and easy and objective result interpretation (the presence of a DNA fragment of specific size indicates the presence of the pathogen). Because of this great potential, over the past 10 years many PCR-based assays have been reported for seed borne pathogens.

Rapid-Cycle Real-Time PCR:

As previously mentioned, commercial and government seed testing agencies have been slow to adopt PCR-base seed detection assays. This has been due in part to the cost of the equipment and consumables, and level of technical expertise required to conduct the assay. Additionally, the risks of cross contamination and the need for post- PCR steps such as gel electrophoresis, have made the technique unattractive. Recent advances in PCR, in the form of rapid-cycle real-time PCR promise to eliminate many of these barriers and make PCR more accessible for seed detection. With real-time PCR, DNA amplification is coupled with the production of a fluorescent signal that increases proportionally with the numbers of amplicons produced. The fluorescent signal is monitored on a computer in real time and provides an indirect visual representation of DNA amplification.

DNA Chip / Microarray Technology

DNA chips or microarrays represent another DNA-based detection assay that may be applied to test seeds for pathogens. This relatively new technology relies on the unique ability of nucleic acid molecules to hybridize specifically with molecules with complementary sequences. With DNA chip technology, oligonucleotide probes are attached to small (approximately1 cm^2) glass or silica-based surfaces (chips).

The power of this technique lies in the fact that hundreds to thousand of oligonucleotides can be attached to specific, locations on each chip. These oligonucleotides can be complementary to DNA sequences that are unique to certain microorganisms and hence, can be used detect pathogens in seed samples. To apply DNA chip technology, DNA or RNA must be extracted from the sample being tested and amplified. The amplified DNA is digested into smaller fragments that are then labeled with fluorescent markers and hybridized with oligonucleotides fixed to the DNA chip.

After hybridization, the chip is washed thoroughly and fluorescence, which is directly proportional to the amount of nucleotide retained, is measured. If the DNA from the pathogen of interest in present in the seed sample, then the oligonucleotide probe at the position on the chip that corresponds to that pathogen will display fluorescence.

Detection / Testing of GMO Crops

A range of transgenic plants are already approved or under approval internationally. This poses a problem for inspection authorities to ensure that the non-approved GMOs (genetically modified organisms) are not imported into the country either as constituents of feed and food products or as "GMO-impurities" in products of the food chain. Thus it is necessary to distinguish GMOs from non-GMOs.

Level of detection:

1. **Detection:** to determine whether a product is GM or not. For this purpose, a general screening method can be used. The result is a positive/negative statement.

2. **Identification:** to find out which GM crop or product are present and whether they are authorized or not in the country.

3. **Quantification:** If a product has been shown to contain GMO(s), the next step is to assess compliance with the 1% threshold level (or the 0.3 or 0.5% level, respectively for seeds) by the determination of the exact amount of each of the GMOs present in the sample.

Methods of testing GMOs

Lateral flow strips: Lateral flow strips are widely used as screening devices for testing of large bulk samples of agricultural commodities for the presence of transgenic proteins. Unfortunately, these strips generally are not very sensitive, and their ability to discriminate between authorised events and UGM expressing the same trait is usually limited.

Procedure:

- ✓ Collect leaf/seed and extract sample
- ✓ Place the strip into the extraction tube and allow the strip for 10 minutes to develop lines
- ✓ Than the sample migrates up the strip by capillary action.
- ✓ Sample passes through a zone of reagent that contains antibodies labelled with colloidal gold.
- ✓ This labelled antibody binds to the GM protein, if present in the sample
- ✓ The antibody-protein complex then continues to move up the strip until it reaches a second zone of antibodies which is immobilized on the test strip

✓ The test strip also contains an immobilised control zone that binds a control complex that is present in the extraction solution and also produces a visible line.

Inference: If there is no target GM protein present, only a single line will form at the control zone. If any GM protein is present, two lines will be developed that indicates the test is positive and based on this one can estimate the purity.

Protein detection methods: Protein based techniques are also often referred to as immunological techniques because the detection is based on the immunological principle of conjugation between an antigen (the target) and an antibody (the probe specific to the antigen). The most commonly applied protein based methods are

1. Lateral flow strips
2. ELISA
3. Western blot method

DNA based amplification methods: DNA can be amplified with several techniques. The most common technique is the polymerase chain reaction (PCR) technique, employing a thermostable DNA polymerase. Other techniques can be performed at ambient (room) temperature (isothermal; not further discussed here). All the amplification techniques involve denaturation of the double stranded nucleic acid followed by annealing of a short oligonucleotide (primer) and primer extension by a DNA polymerase. The nature of the primer, the number of primers involved, etc. varies from method to method.

PCR Methods for Screening:

a. ***Construct-Specific, Qualitative PCR Methods****:* Construct-specific methods are designed to confirm the presence or absence of a specific sequence that is not found in nature. Amplification of a trait-specific sequence such as a modified gene sequence, or the junction between a promoter and gene sequence provides for more information than a screening method.

b. **Event-Specific, Qualitative PCR Methods***:* For unequivocal identification of a specific GM event, the PCR product to be amplified must span the junction between the construct and the plant genomic DNA. This last style of PCR, known as event-specific PCR, is the most informative, as the junctions at each end of the construct are always present in two copies per genome (one copy on each chromosome) in approved GMOs and are absolutely specific for that event.

c. **Quantitative PCR Methods**: PCR is most commonly used to provide a relative rather than absolute quantification for the level of GMO in a sample. Relative quantification is achieved by amplifying two DNA sequences and calculating the ratio between the amounts of amplified products obtained.

d. **Southern blot:** The method involves fixing isolated sample DNA onto nitrocellulose or nylon membranes, probing with double-stranded (ds)-labeled nucleic acid probes specific to the GMO, and detecting hybridization radiographically, fluoremetrically or by chemiluminescence.

e. **Microarrays:** Microarray technology (DNA chip-technology) has been developed in recent years for automated rapid screening of gene expression and sequence variation of large number of samples. Microarray technology is based on the classical DNA hybridisation principle, with the main difference that many (up to thousands of) specific probes are attached to a solid surface.

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Genetic Purity Testing Through Molecular Techniques

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Introduction

Biochemical and Biotechnological approaches have been used for developing and improving the crop plants for improved seeds with improved nutritional quality like Golden rice, Golden mustard and nutraceuticals, for better oil-content, with more by-products like glycerine, oleosins, etc. and for hybrid seed production.

Biochemical components like gibberellins, enzymes like alpha amylase and other hydrolytic enzymes present in the seeds of cereal grains and grasses stimulate the mobilizationof food and mineral elements in seed storage cells. The level of these biochemical constituents evaluates the seed quality, its germination and viability. These can be quantified or tested using various biochemical techniques. Seed germination, seedling vigor and viability tests are performed based on biochemical approaches.

Studies related to genetic stability of the seed are based on evaluation of morphological traits, isoenzyme patterns and DNA markers like RFLP, etc. that serve as biotechnological tools.

In this paper an effort has been made to list and emphasize the major biochemical and/or biotechnological tools and techniques which are/can be used for seed quality evaluation.

Parameters for Evaluation of Seed Quality

The seed quality for various types of seeds are tested for the following attributes using various biochemical, biotechnological and bioinformatics tools.

A. Physical parameters

1. Seed size
2. Seed colour
3. Seed coat thickness
4. Seed surface

B. Physiological parameters

1. Seed germination
2. Seedling vigor
3. Seed coat protein
4. Seed viability
5. Level of biochemical constituents
6. Isoenzymic pattern
7. Genetic makeup

Ideally seed quality tests should efficiently differentiate between poor and good seed lots in a short period and produce data that correlate with stress and planting conditions. Various genetic factors lead to differences in the chemical composition of seeds both within species and varieties. This is taken care of by the biochemical techniques like

determination of protein, lipid and carbohydrate followed by type of protein analysis using standard methods.

For seed quality testing, both genetic quality and seed health testing two highly sensitive and specific biotechnological tools are being used. These include ELISA- a serological test and polymerase chain reactor, (PCR)- a DNA test. For genetic quality tests, ELISA uses specific antibodies to detect protein products resulting front an inserted gene and in seed health testing; the specific antibody detects a targeted plant pathogen protein. PCR is used to increase specificDNA fragments of either the inserted gene in genetic quality testing or specific pathogen ONA in seed health testing. Both of these techniques are very efficient.

With the advent of genetically modified (GM) seeds the varietal verification and GM seeddetection in seed lots are the most important seed quality parameters, A number of advanced techniques are available for seed testing. However, their applications require appropriate technical competence and laboratory infrastructure. The use of reliable and economical methodology for variety verification and detection of GM seeds are becoming more important for all countries especially for those exposed to international seed trade.

The varietal identification, hybrid purity determination as well as quantitive and qualitative seed detection are important for food safety besides increasing the seed quality and develop links between seed analysts and researchers.

Imaging techniques for physical and seedling vigor

The physiological parameters of seed is assessed by use of imaging techniques in a non-destructive manner. It is advantageous as it provide instantaneous visualization of heterogeneity. The most common techniques in use are Thermography, Bioluminiscence Imaging, Reflectance Imaging, Flourescence Imaging, Magnetic Resonance Imaging and Multispectral Imaging.

For testing the seedling vigor of seeds, tests like Cold test, Accelerated aging test and imaging tests are performed. The Seed Vigour System (SVIS) is now being used as an alternative to traditional vigour tests, which require longer time and are less reliable when compared with field tests.

Use of Embryo culture for germination

Embryo culture technique can be used to determine the germination potential of seeds, wherever normal germination tests cannot be used for the seed lots of interest. Such a situation arises when seeds require a period of after-ripening or a brief storage to overcome dormancy and the seed has to be used immediately after this period. Germination of embryos is more reliable test for seed germination than seed viability tests based on staining methods. This is useful when seeds grown in one region is transported to another region for use in the next season. In embryo culture technique young embryos are removed from developing seeds and are placed in suitable nutrient medium to obtain seedlings. The cultured embryos generally do not complete development, but germinate prematurely to give rise to seedlings, called as precocious germination.

Gel Electrophoresis

Gel electrophoresis is employed to check progression of a restriction enzyme digestion, to quickly determine the yield and purity of a DNA isolation or PCR reaction, and to size fractionate the DNA/RNA molecules, to know the molecular weights of proteins, their subunits, for isozymes studies, etc. In a nutshell it is a technique of verification and most of the advanced biotechnological techniques are dependent on it. The variations of the gel electrophoresis technique include:

- Agarose gel electrophoresis (used for DNA/RNA)
- Polyacrylamide gel electrophoresis (used for protein)
- SDS-PAGE (used for protein subunits)

Molecular Markers of Seed Quality

Many genes have been described that are involved in germination, dormancy and other parameters for seed quality. Provided the expression pattern of these genes is consistent, mRNA abundance could be used as a marker for seed quality of a range of species. Potential molecular markers can be subdivided into several classes.

The first class of molecular markers would be genes involved in metabolism, which are up-regulated upon imbibition Expression of these genes would be expected to indicate the viability of a seed. For example, if induction of ribosomal proteins correlates with imminent radicle protrusion and low expression with dormancy, then expression of these ribosomal proteins could serve as a molecular marker for germination.

The second class of molecular markers would be genes involved in dormancy or quiescence, which are up-regulated when seeds are dormant and down-regulated when completion of germination is imminent.

Other classes that may contribute to seed quality are genes involved in seed development, desiccation tolerance, and longevity.

In order to find these (widely applicable) molecular markers, target genes (cDNAs) arecloned in a species. Expression patterns are assessed using a model species and in a rangeof other species using Northern analysis, in order to predict applicability. The genes with the highest interest are those that are highly conserved. This high degree of homology provides a certain guarantee for its applicability. Apart from Northern analysis, semi-quantitative RT-PCR with gene specific primers is performed to complement Northern analysis.

Polymerase Chain Reaction (PCR) Amplification Test

For seed health tests, the seed lot of samples are tested for seed-borne pathogens using the Polymerase Chain Reaction (PCR), a method that is able to extract, detect and identify pathogens based on fragments of their genetic information.

RT-PCR Testing

The Reverse Transcriptase Polymerase Chain Reaction is a technique used in genetic studies that allows the detection and quantification of mRNA. It is a very sensitive method that shows whether or not a specific gene is being expressed in a given sample. RT-PCR is a very important test in the field of seed genetic studies because it gives researchers a mechanism to test whether any specific gene is turned on (active) or turned off (inactive). This allows researchers to identify the benefits of a genetically-modified

seed with respect to their "natural" counterparts and search for any significant differences in which genes are expressed in the two types of seeds.

RT-PCR is used to locate and quantify known sequences of mRNA in a sample. The first step in RT-PCR uses reverse transcriptase and a primer to anneal and extend a desired mRNA sequence. If the mRNA is present, the reverse transcriptase and primer will anneal to the mRNA sequence and transcribe a complimentary strand of DNA. This strand is then replicated with primers and Tag Polymerase, and the standard PCR protocol is followed. This protocol copies the single stranded DNA millions of times in a small amount of time to produce a significant amount of DNA. The PCR products (the DNA strands) are then separated with agarose gel electrophoresis. If a band shows up for the desired molecular weight, then the mRNA was in fact present in the sample, and the associated gene was being expressed.

RT-PCR shows us whether or not a specific gene is being expressed in a sample. If agene is expressed, its mRNA product will be produced, and an associated band will inthe final agarose gel with the correct molecular weight for the gene This is used for transgenicseeds to identify if a gene that has been transplanted and is present in the hybrid seed hasundergone any changes. Also, RT-PCR can quantify exactly how active the gene is in the sample.

ELISA Testing

ELISA (Enzyme-Linked Immunosorbent Assay) is a diagnostic test used to determine if proteins from a particular suspect plant pathogen (virus, bacteria. or fungus) are present in a sample. ELISA testing uses antibodies that detect specific proteins from the target pathogen. First, the wells of a micro-titre plate are coated with the antibody. Then a sample is added to the wells and if the target proteins from the pathogen present, they bind to the antibody. A second antibody with an attached enzyme is added and it binds to the pathogen protein. A chemical substrate is then added to the wells, which then reacts to the enzyme to produce a color change. A color change indicates that a sample is positive for the protein and therefore the pathogen of interest.

Testing viability of Virus by Bioassay

The bioassay is a method of detecting viable (active) virus particles by using a host plant that gives a specific reaction when inoculated with the target virus. Tobacco is commonly used as a "host plant" to detect Tobacco Mosaic Virus (TMV), Tomato Mosaic Virus (ToMV), and Pepper Mild Mottle Virus (PMMV) by eliciting a rapid lesion called the "Hypersensitive Response (HR)".

The Seed sample is ground in a buffer solution and inoculated on fully expanded tobacco leaves. The plant leaves are gently wounded during the inoculation to allow the virus particles to more easily enter the plant. After approximately seven days, if there are no lesions, the sample is considered to have no evidence of the targeted virus.

Another type of bioassay utilizes a 'systemic reaction' (those that appear on leaves other than the one that is inoculated) to detect viral pathogens that can move systemically through the plant. These types of bioassays are more time consuming to conduct due to the length of time that is required for symptom expression after inoculation.

Analysis of Seed DNA/RNA

Techniques have been devised to identify specific nucleic acids in these complex mixtures

- Southern blotting - DNA
- Northern blotting - RNA

These techniques are different from Western blotting which is used to analyse **PROTEINS** which have been immobilizedon nitrocellulose/nylon filters. Proteins which have been separated by polyacrylamide gel electrophoresis (**PAGE**) are transferred to nitrocellulose/nylon filters and the filter is probed with antibodies to detect the specific protein - similar to the method used for expression library screening

Southern blotting evaluates a seed for

- Presence of a particular gene and number of its copies present in the genome of theseed under test
- The degree of similarity between the chromosomal gene and the probe sequence
- Whether recognition sites for particular restriction endonucleases are present in the gene. By performing the digestion with different endonucleases, or with combinations of endonucleases, it is possible to obtain a restriction map of the gene i.e. an idea of the restriction enzyme sites in and around the gene- which will assist in attempts to clone the gene
- Whether the cloned seed has undergone any changes upon storage or in field.

Northern blotting tests for seed evaluation express

- Differential expression patterns of a particular gene

a) In which tissue part of seed it is expressed
b) At what stage of seed development it is expressed
c) If expression changes under differing conditions/treatments

- The quantity of the mRNA as the blots can be quantitated accurately by radioactive counting
- Whether a genomic DNA probe has regions that are transcribed

Both Southern and Northern blots can detect very small amounts of nucleic acids. Blots with both RNA and DNA from different seeds can inform us on the conservation of genes among them.

Conclusion

The seed characterization, germination percent, vigor tests and the genetic makeup constitute the basis of seed evaluation tests. The various biochemical and biotechnological techniques involved with the seed studies require a well-developed laboratory infrastructure and expertise in various techniques. A standardization of these methods in labs for cross-checking the results as per the type of seed is mandatory. The appearance of the seed and its germination with the stages leading to growth and the biochemical conversion of storage reserves as a measure of seedling vigor explains the seed quality and if it has undergone any changes ors deterioration. Seed-informatics has led to an amalgamation of available data from biotechnology and various other techniques bringing forth a new avenue for utilization of the generated data for accurate comparison of a seed with its hybrid or a seed differing in type andorigin. The future holds promises in

advancement of seed technology by the proper use of computational biology in phases or phaseomics that biochemistry, biotechnology, molecular biology and genomics, proteomics, transcriptomics and metabolomics. The bond between plant breeders and farmers can affirmed by evaluation of seeds for better confidence and product yield.

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