Advance Training
on
"Seed/DNA Testing"

Training Manual

GOVERNMENT OF INDIA
Ministry of Agriculture & Farmers Welfare
Department of Agriculture & Farmers Welfare
NATIONAL SEED RESEARCH & TRAINING CENTRE
GT Road, Collectry Farm`, Varanasi- 221106
Phone: 0542-2370222, Fax: 0542-2370298
E-mail: dir-nsrtc-up@nic.in, Website: www.nsrtc.nic.in

ADVANCE TRAINING
ON
SEED / DNA TESTING
(DECEMBER 18-22, 2023)

Course Director

Sh. Manoj Kumar, IAS
Director

Training Coordinators

Sh. Javesh Kumar
Junior Seed Analyst

Smt. Ekta Kumari
Senior Seed Analyst

Organized by:

Government of India
Ministry of Agriculture& Farmers Welfare
Department of Agriculture & Farmers Welfare

NATIONAL SEED RESEARCH AND TRAINING CENTRE
VARANASI-221106 (UTTAR PRADESH)
Tel: 0542-2370222, Fax: 0542-2370298
E-mail: dir-nsrtc-up@nic.in
Website: www.nsrtc.nic.in

Publication No. NSRTC/2023/007

ADVANCE TRAINING
ON
SEED / DNA TESTING
(DECEMBER 18-22, 2023)

Compiled & Edited by:

Sh. Javesh Kumar, Junior Seed Analyst

Mrs. EktaKumari, Senior Seed Analyst

Er. M. K. Vishwakarma, Seed Processing Engineer

Dr. M. P. Yadav, Seed Technologist

Dr. A. K. Verma, Senior Seed Analyst

Sh. Anil Varma Nalla, Junior Seed Analyst

NATIONAL SEED RESEARCH AND TRAINING CENTRE

VARANASI-221 106 (UTTAR PRADESH)

Advance Training
On
Seed/DNA Testing
(December18 – 22, 2023)
CONTENTS

*****

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 221 106 (उ.प्र.)

सत्यमेव जयते
Government of India

GOVERNMENT OF INDIA
NATIONAL SEED RESEARCH & TRAINING CENTRE
Ministry of Agriculture & Farmers Welfare
Deptt. of Agriculture & Farmers Welfare
G.T. Road, Collectry Farm,
P.O. Industrial Estate, Varanasi-221106 (U.P.)

FOREWORD

Seed is a basic input for an efficacious crop production and Seed testing is the science of evaluating the quality of seeds to determine their value for planting. Use of high quality seeds result higher yields. It is estimated that quality seed can contribute up to 15-20% increase in total production, which can be further increased with efficient management of other inputs depending upon the seed crops. Until and unless, the seed varietal and physical purity, germination, moisture and other seed standards are maintained, production programme cannot be successful. To maintain these quality standards, seed testing is most important.

In this context, National Seed Research and Training Centre, Varanasi has organized five days Advance Training on "Seed/DNA Testing" during 18-22 December, 2023. The main objective of this Advance Training is to update the knowledge, skills and to provide hands on experience to Seed/DNA testing personnel. The Training will provide a platform to update the knowledge and skills of the Seed Analysts and Seed Testing Officers who are working in various Seed Testing Laboratories across the country in order to obtain uniformity in seed testing and seed quality regulation at national level.

Accordingly, the Training programme has been design and the experts who have expertise in seed/DNA testing and seed quality related issues are invited. The Training module is designed to provide participants with the updated information's so as to effectively assess seed quality attributes, understand better seed quality management and improve the present system to meet domestic and international standards as per the need of hour.

I hope that this training module will be beneficial to all the participants involved in Seed Testing.

(Manoj Kumar, IAS)
Director

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 visions and mission's 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 inabout 2.5 hectares of land andremaining 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 & 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

Seed Sampling: Principles and Procedures

Dr. M. P. Yadav
Seed Technologist, National Seed Research & Training Centre, Varanasi

Seed sampling is the process of obtaining the representative portions of small quantities of the seed from the seed lot. The process itself is a highly technical and it is the pre-requisite of seed testing. The analysis results obtained on the sample tested in the seed testing laboratory may cause the rejection of the seed lot for distribution or further multiplication, certification or may serve as evidence in the Court of Law against the seller of faulty seeds. It is neither physically possible nor practicable to test the entire quantity of the seed lot. Accordingly it is essential that the sample drawn from the seed lot must be representative to avoid problems in seed certification and seed law enforcement. It is customary that the analysis results on the sample tested in the seed testing laboratory should reflect the quality of the whole lot from where the sample was drawn.

Principles of Sampling:

Samples are derived from different portions of a seed lot and mixed to obtain a sample of required quantity representing the seed lot in true sense. From this composite sample, small portion of required quantity is obtained in such a way that even after reduction, it represents the seed lot. In each and every stage thorough mixing and dividing is necessary.

Seed Lot:

A seed lot is a specified quantity of the seed of one cultivar, of known origin and history and controlled under one reference number (lot number). It is an uniformly blended quantity of seed either in bag or in bulk.

Equipment and Materials: Trier, plastic tubs, bags, balance, seed divider, sticker and labels.

Trier: It is required to draw the primary sample from the seed lot stored in bags or containers. Two types of triers are required for sampling *Stick and Nobbe trier.*

Seed divider:

It is equipment used for getting desired quantity of true to the type sample for submission in laboratory for individual test. Three types of divider are used in seed testing Boerner *type divider* (conical divider), *Soil type divider* and *Gamet type divider* (centrifugal divider).

Sampling in processing plant

1) **Primary sample:**

It is a small quantity of seed taken from one point of the processed lot. The seed lot is arranged to approach conveniently up to individual container. Primary samples are drawn from different portions and depth by inserting the stick Trier with the closed slot diagonally in the seed bag or container up to desirable depth with minimum damage to seed. The flow of seed is facilitated in the tube by opening and closing of the slot. Finally, the trier is withdrawn with closed slot and collected sample is transferred to a container.

Stick Trier is inserted into a bag up to a desirable depth at an angle of 30 degree with the hole present at the pointed end facing downwards. The spear is withdrawn gently, so that, equal quantity of seeds enter into the hole from centre to the side of the bag. The point of insertion is closed with the help of a sticker or by running across the trier on the hole a couple of times in opposite direction. Minimum number of primary samples should be taken as per Table 1. and 2. The quantity of seed drawn in one primary sample depends on the sampling intensity, size of submitted sample and seed lot size of crop.

2) **Composite sample:** Primary samples drawn from different places of a lot are mixed and the mixture is known as composite sample. The size of composite sample should be 10 times more than the required submitted sample.

3) **Submitted sample**: The required quantity of seed, which is sent to seed testing laboratory, is known as submitted sample. The weight of the submitted sample varies accordingly to the kind of seed or the kind of test required. (Table 1 and 2). To prepare a submitted sample, the composite sample is mixed thoroughly and reduced up to required quantity with the help of seed divider or by repeated halving method.

Category of seed sample:

Mainly three categories of samples are received in the seed testing laboratory based on their usages. Viz.

a) Service samples
b) Certification samples
c) Enforcement/legal/official samples

Service samples:

These are the samples drawn from the farmer stored stock / dealers by extension workers or by the dealer/farmers themselves to know the quality of the seed for further immediate use. The result obtained on these samples is generally utilized for sowing or labeling purpose. The sample should contain the necessary information for documentation (sample slip). Non notified laboratories can also test these categories of seed samples.

Certification sample:

The samples drawn submitted to the seed testing laboratory by the authorized official from seed certification agency for certification purpose. Such seeds are tested in the seed testing laboratory to know whether they confirmed to the seed certification standard prescribed. Only notified seed testing laboratories are authorized to test the certification samples.

Seed law enforcement sample:

For seed quality regulation at distribution and marketing level these sample are drawn from sale/stock point by the notified seed inspectors in their respective jurisdictions as per the provisions of the section 14 (1) a, b Seeds Act 1966. These samples are also know as quality control samples and are tested only in notified: Seed testing laboratories. These samples are tested by the authorized or notified seed analyst as per the procedure laid down in Seeds Act 1966 and Seed Rules 1968.

Separate sample for determination moisture:

The seeds are hygroscopic in nature and tend to absorb atmospheric moisture when exposed. Therefore when the seed sample is to be taken for moisture content a separate seed sample of 100 gram (for species that require grounding) and 50 gram (for other species) in a polythene bag (700 gauge)/ moisture proof bag is to be apportioned, tightly secured and be submitted along with the submitted sample bag.

Sampling situations:

Seed sample are required to be drawn before or during processing and after bagging or packing operations. Seed may be stored in the form of heaps, in the storage bins/gunny bags / cloth bags, paper packets/pouches or moisture impervious containers such as laminated aluminum foils, sealed tins etc.

General principles of sampling:

1. Sampling should be carried out only by persons trained and experienced in seed sampling.
2. The seed lots shall be so arranged that each individual container or part of the lot is conveniently accessible. Upon request by the sampler, the owner shall provide full information regarding the bulking and mixing of the lot. Sampling may be refused when there is definite evidence of heterogeneity.
3. The size of the seed lot should also not exceed to maximum seed lot size prescribed in the rules, subject to a tolerance of 5%
4. Seed sampler may request the producer to get some bags emptied or partially emptied to facilitate sampling. The bags may then be refilled. This may be

necessary since it is impossible to obtain sample deeper than 400 mm, i.e. from the lower layer in bags and bins.

5. The sampler should determine that all seed bags sampled are identified as belonging to a single lot, either by a label or stencil mark on the bag
6. The sampler must sample the minimum requisite number of bags from the seed lot in accordance with the sampling intensity.
7. Care must be exercised in reducing composite samples. Careless splitting of the sample cannot be expected to produce two similar portions.
8. Any seed know to have been treated with a poisonous fungicide should be identified so that the person who subsequently may handle the sample will be informed of the potential hazard.
9. While taking samples from machine sewed cotton bags, a few stitches at one of the top corners can be broken and then this break can be closed with a hand stapling device, after the contents of the bag have been sampled.
10. The sample drawn should not be less than the weight of submitted sample prescribed in the rules.

Table 1: Sampling intensity for a seed lot stored in container

Number of container	Sampling intensity
up to 5	Each container, at least 5 Primary samples
6 - 30	Sample 5 Containers or at least one in every three containers, Whichever is the greater
31 - 400	Sample 10 Containers or at least one in every 5 containers, Whichever is the greater
401 or More containers	Sample 80 Containers or at least one in every 7 containers, Whichever is the greater

Table 2: Sampling intensity for seed stored as bulk

Lot size (Kg)	Sampling intensity
up to 500	At least 5 primary Samples.
501 - 3,000	One primary sample for each 300kg, but not less than 5 primary samples.
3,001-20,000	One primary sample for each 500 kg, but not less than 10 primary samples.
20,001 and Above	One primary sample for each 700 kg, but not less than 40 primary samples.

Dispatch of submitted sample:

Sample should be dispatched to the seed testing lab as early as possible providing all the details like date of sampling, number of processing plant, crop, variety, class of seed, lot number, lot size / Quantity of seed in lot (kg) Senders Name and Address etc. and Tests required: 1) Purity (2) Germination (3) Moisture, apart from this sample, two reference samples are also prepared by the same method. One reference sample is stored by the office and second by producer. Office sample of seed lot passed in seed testing is stored for two years.

Sampling in seed testing lab:

The submitted sample received in seed testing lab is registered and designated by a code number. Submitted sample is tested for determination of seeds of other crop, weed, objectionable weeds, objectionable diseases and other distinguishing varieties by number. Three working samples of the submitted sample, which passes the seed certification standard by number are prepared. Each working sample consists of at least 2500 seeds (Table 3).

Preparation of working sample:

Mechanical divider: As described for preparation of submitted sample.

Repeated halving method: As described for preparation of submitted sample or the seed is poured on a clean smooth surface and shaped as a mound after thorough mixing. Mound is divided into two halves, each half is again halved, each portion is again halved giving total 8 portions. Alternate portions are combined i.e. 1st and 3rd of first row and 2nd and 4th of second row. The remaining portion is kept in a pan and the process is repeated to obtain required size of the working sample.

Random cup method: Six to eight small cups of equal size and shape are arranged at random on a tray. The seed is poured uniformly over the tray. The seeds, which fall into the cups, are collected as working sample. This method is useful for the crops with small seed size but not for chaff and round seeds.

Spoon method:

The seeds are poured evenly in one direction over the tray. If required, seed can be poured second time in opposite direction. Shaking of the tray is avoided, small quantity of seeds are collected with the help of spatula from minimum 5 random places to make a working sample of required quantity. The working sample is stored in paper bag marked with code number, name of the crop and purpose.

Table 3: Size of submitted and working samples required for different crops

Crop	Submitted sample (g)	*Working sample (g)*
FIELD AND FODDER CROPS		
Wheat, oat, triticale	1000	0120
Sorghum	0900	0090
Pearl millet	0950	0015
Italian millet	0090	0009
Kodo millet	0080	0008
Linseed, jute, common millet	0150	0015
Fieldpea, maize	1000	0900
Lentil	0600	0060
Chickpea, groundnut	1000	1000
Pigeonpea	1000	0300
Horse gram, moong bean	1000	0400
Grass pea	1000	0450
Castor, soybean	1000	0500
Rice, rajmash, urid bean	1000	0700
Sunflower	1000	0200
Safflower	0950	0090
Cotton	1000	0350
Gueina grass, Setaria grass	0025	0002
Marvel grass	0030	0003
Brassica juncea, taramira	0040	0004
Lucerne, Indian clover	0050	0005
Egyptian clover, finger millet, buff grass	0060	0006
VEGETABLE CROPS		
Celery	0025	0001
Chinese cabbage, parsley	0040	0004
Carrot, lettuce	0030	0003
Tomato	0015	0007
Turnip	0070	0007
Onion	0080	0008
Brassica olerecea all varieties	0100	0010
Chilli, egg plantl	0150	0015
Cucumber, musk melon	0150	0070
Spinach	0250	0025
Radish	0300	0030
Pumpkin	0350	0180
Coriander	0400	0040
Fenugreek	0450	0045

Sugar beet	0500	0050
Cluster bean, asparagus	1000	0100
Okra	1000	0140
Water melon, sponge gourd	1000	0250
Ridge gourd	1000	0400
Bitter gourd	1000	0450
Bottle gourd	1000	0500
Indian bean	1000	0600
French bean and all squashes	1000	0700

*****

Advance Techniques for Estimation of Moisture Content in Seed Sample

Nakul Gupta
ICAR-Indian Institute of Vegetable Research, Varanasi 221305, U.P.

The amount of moisture in a seed has a direct impact on every physiological component of seed quality. It has been demonstrated that there are correlations between moisture content and seed maturity, ideal harvest time, storage longevity, cost-effectiveness of artificial drying, damage from heat, frost, fumigation, insects, and pathogens, mechanical damage, and seed weight. Seed moisture measuring techniques suitable for the purpose are required in both research and business because seed moisture and its management affect so many physiological quality variables crucial to quality control.There is no one method of moisture determination that is suitable for all products or circumstances, as evidenced by the extensive literature on the determination of moisture content in a variety of items. The same is true for assessing the moisture content of grains and seeds. The most effective method for evaluating seed moisture depends on the chemical make-up and seed structure, moisture content level, level of accuracy and precision needed, and time, technical know-how, and cost restrictions.

The chemical makeup of the seed and the interactions of the seed with water impose challenges in the measuring of moisture in seeds. The strength of water retention in the seed varies, ranging from free water to water that is chemically bonded. The bonded water is harder to remove while drying while the free water is removed quickly and readily. Free water can be removed using the standard heat of vaporization, while bonded water needs more heat to be removed.Equilibrium moisture curves are commonly used to characterise the water-binding connections in seeds (water-sorption isotherms). Hygroscopic seeds release or absorb water from the air around them until the relative humidity (RH) of the air and the moisture content of the seeds are equal.

Calculating the moisture content of seeds

Numerous physiological seed quality characteristics that are crucial to seed quality are influenced by how seed moisture is managed. In trade and research, accurate seed moisture assessment is required. The best technique for checking for moisture depends on:

- Chemical makeup of seeds
- Seed morphology
- The degree of accuracy and precision necessary
- Limitations of time
- Technical expertise and budget

Calculating the moisture content of seeds

The ideal device might be one that is applied to all seeds, measures moisture content from 0 to 100%, is repeatable, needs little training, and is inexpensive. One cannot blend all of

these. However, techniques for determining the moisture content of seeds can be roughly divided into two categories:

1. Direct method
2. Indirect method

Direct method: In this group, the seed moisture content is quantified through direct weight change or loss. The methods are as following.

1. Desiccation method
2. Phosphorus pentaoxide method
3. Oven-drying method
4. Vacuum drying method
5. Distillation method
6. Karl Fisher's method
7. Direct weighing balance
8. Microwave oven method

Indirect method: These estimates are not very accurate; however, they are quick and easy to use. These are typically utilized in seed processing facilities. These gauge other physical characteristics like the moisture content of the seed's moisture resistance or electrical conductivity. Seed moisture meters are used to measure readings, which are then converted into the seed moisture content for each species using calibration charts against the conventional air-oven method or a fundamental reference method.

Above all, the Karl-Fisher method has been regarded as the most precise and fundamental reference method for establishing standards for other methods of determining seed moisture. The sole feasible technique recognized by the International Seed Testing Association (ISTA) and other organizations for use in routine seed moisture analysis in a seed-testing laboratory is the constant temperature oven drying method.

Constant temperature oven drying method

The two categories of the constant temperature oven drying method are generally used:

1) Low Constant Temperature Oven Method
2) High Constant Temperature Oven Method

Low constant temperature oven method: This method has been recommended for seed of the species rich in oil content or volatile substances (Table I). In this method, the pre-weighed moisture bottles along with seed material are placed in an oven maintaining a temperature of 103°C. Seeds are dried at this temperature for 17 ± 1hr.The relative humidity of the ambient air in the laboratory must be less than 70 percent when the moisture determination is carried out.

Table1: Species for which the low constant temperature (103°C) oven method be used

S.N.	Species	Sr. No.	Species
1.	Allium spp	**2.**	Linumustatissimum

3.	Arachis hypogea	**4.**	Raphanus sativus
5.	Brassica spp	**6.**	Rlcnuscommunis
7.	Camelina sativa	**8.**	Sesamum indicum
9.	Capsicum spp	**10.**	Sesamum orientale
11.	Glycine max	**12.**	Sinapsisspp
13.	Gossypium spp	**14.**	Solanum melongene

High constant temperature oven method: The process is the same as described previously, with the exception that the oven temperature is kept between 130° and 133°C. For maize, the sample is dried for four hours, for other cereals it is dried for two hours, and for other species it is dried for an hour (see alsoTable2). The relative humidity of the ambient air in the laboratory during the moisture determination is not specifically required for this procedure.

Table 2: Species for which high constant temperature (130 to 133°C) used

S.N.	Species	S.N.	Species
1.	Agrostis spp	**2.**	Citrullus lanatus
3.	Loliumspp	**4.**	Phaseolus spp
5.	Alopecurus	**6.**	Cucumis spp
7.	Lotus spp	**8.**	Phelumspp
9.	Pratensis	**10.**	Anethumgraveolens
11.	Cucurbita spp	**12.**	Lupinusspp
13.	Pisum sativum		

Table3: Species for which grinding is obligatory

S.N.	Species	S.N.	Species
1.	Avenaspp	**2.**	Gossypium spp
3.	Phaseolus spp	**4.**	Triticum spp

Essential equipment and supplies

1. Constant temperature precision hot-air electric oven
2. Weighing bottles/Moisture containers
3. Desiccator with silica gel
4. Analytical balance capable of weighing up to 1mg
5. Seed grinder/An adjustable grinding mill
6. Heat resistant gloves
7. A brush/A steel brush

Period of seed drying

The recommended drying times for seeds are 17±1 hours at 103°C for low continuous temperatures and 1 to 4 hours at 130°-133°C for high constant temperatures. Cereals and/or other millets should be dried for two hours, followed by the remaining species for one hour. It is recommended that seeds with a high oil content or volatile compounds be

dried for 17± 1 hours at a low, steady temperature. When the oven starts up again to maintain the proper temperatures, the drying process starts.

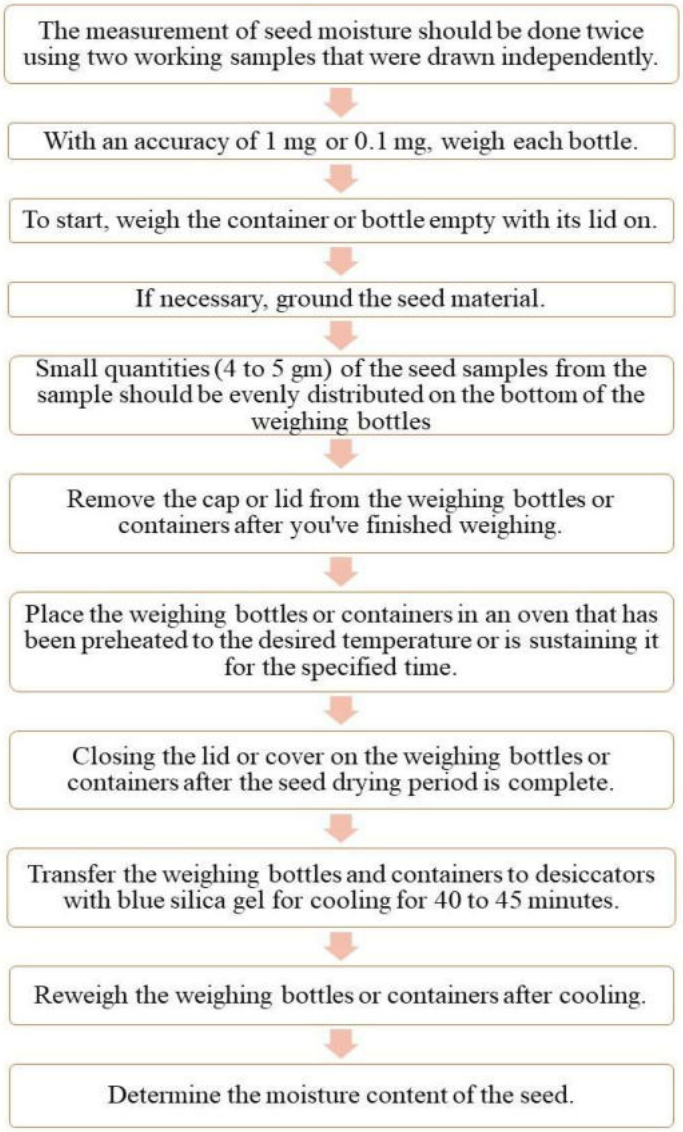
Sample

The ISTA guidelines advise using two replicates, each containing 4 g of seed, to measure the seed's moisture content. To prevent needless waste of priceless biological resources, this seed sample weight may be changed to 0.2 to 0.5 gram per replication, with exact weighing, for use in seed genebanks.

Apparatus: The following apparatus is required, depending on the method used.

1. Grinding mill;
2. Electrically heated oven;
3. Containers;
4. Desiccator
5. Balance;
6. Sieves;
7. Cutting tool.

Procedure:



Calculation of results

To one decimal place, the moisture content is calculated as a percentage of weight (fresh weight basis), by using of the formula:

$$\text{Seed Moisture Content (\%)} = \frac{\text{Loss of weight}}{\text{Initial weight}} * 100 \text{ or } \quad \frac{M2-M3}{M2-M1} * 100$$

Where,

$\mathbf{M_1}$ is the weighing bottle's or container's weight in grams with the cover on.

$\mathbf{M_2}$ is the weight, before drying, of the weighing bottle or container with a cover on it and seeds.

$\mathbf{M_3}$ is the weight of the weighing bottle or container after drying the seeds and adding the lid.

Note: Two replicate measurements of the seed moisture must be made, with accurate weighing (i.e., up to three decimal places) carried out using lightweight weighing bottles/containers.

Use of tolerances

The outcome is the arithmetic mean of the two independent measurements of seed moisture content for a specific seed sample. According to ISTA regulations, a maximum deviation of 0.2% is advised between two replicates for crop seed species. Repeating the seed moisture determination in duplicate is necessary if the difference between two replicates is more than 0.2%. Because it is virtually hard to meet the duplicate seed difference, According to ISTA regulations, seed moisture in tree or shrub species should be kept to a maximum of 0.2%, with a recommended range of 0.3 to 2.5% between two replicates.

Table-4. Details of methods for moisture determination: agricultural and vegetable seeds

Sr. No	Species	Grinding/ utting	Method to be used	Drying at high temperature (h	Pre-drying requireme
1	Agrostis spp.	No	High	1	–
2	Allium spp.	No	Low	–	–
3	Alopecurus pratensis	No	High	1	–
4	Anethumgraveolens	No	High	1	–
5	Anthoxanthumodorat m	No	High	1	–
6	Anthriscus spp.	No	High	1	–
7	Apiumgraveolens	No	High	1	–
8	Arachishypogaea	Cut	Low	–	To 17 % moisture content or less
9	Arrhenatherum spp.	No	High	1	–
10	Avena spp.	Coarse	High	2	To 17 % moisture content or less
11	Beta vulgaris	No	High	1	–
12	Brassica spp.	No	Low	–	–
13	Bromus spp.	No	High	1	–
14	Camelina sativa	No	Low	–	–
15	Cannabis sativa	No	High	1	–
16	Capsicum spp.	No	Low	–	–
17	Carumcarvi	No	High	1	–
18	Cenchrus spp.	No	High	1	–
19	Chloris gayana	No	High	1	–
20	Cicer arietinum	Coarse	High	1	To 17 % moisture content or less
21	Cichorium spp.	No	High	1	–
22	Citrullus lanatus	Coarse	High	1	To 17 % moisture content or less
23	Cucumis spp.	No	High	1	–
24	Cucurbita spp.	No	High	1	–

25	Cuminumcyminum	No	High	1	–
26	Cynodondactylon	No	High	1	–
27	Cynosurus cristatus	No	High	1	–
28	Dactylisglomerata	No	High	1	–
29	Daucus carota	No	High	1	–
30	Deschampsia spp.	No	High	1	–
31	Elytrigia spp.	No	High	1	–
32	Fagopyrum esculentum	Fine	High	2	To 17 % moisture content or less
33	Festuca spp.	No	High	1	–
34	Galegaorientalis	No	High	1	–
35	Glycine max	Coarse	Low	–	To 12 % moisture content or less
36	Gossypium spp.	Fine	Low	–	To 17 % moisture content or less
37	Helianthus annuus	No	Low	–	–
38	Holcuslanatus	No	High	1	–
39	Hordeum vulgare	Fine	High	2	To 17 % moisture content or less
40	Lactuca sativa	No	High	1	–
41	Lathyrus spp.	Coarse	High	1	To 17 % moisture content or less
42	Lepidium sativum	No	High	1	–
43	Linumusitatissimum	No	Low	–	–
44	Lolium spp.	No	Low		
45	Lotus spp.	No	High	1	–
46	Lupinus spp.	Coarse	High	1	To 17 % moisture content or less
47	Macroptiliumatropur ureum	Coarse	High	1	To 17 % moisture content or less
48	Medicago spp.	No	High	1	–
49	Megathyrsus maximu	No	High	2	–
50	Melilotus spp.	No	High	1	–
51	Nicotiana tabacum	No	High	1	–
52	Onobrychisviciifolia	No	High	1	–
53	Ornithopus sativus	No	High	1	–
54	Oryza sativa	Fine	High	2	To 13 % moisture content or less
55	Panicum spp.	No	High	2	–
56	Papaver somniferum	No	High	1	–
57	Paspalum spp.	No	High	1	–
58	Pastinaca sativa	No	High	1	–
59	Petroselinum crispum	No	High	1	–

60	Phacelia tanacetifolia	No	High	1	–
61	Phalaris spp.	No	High	1	–
62	Phaseolus spp.	Coarse	High	1	To 17 % moisture content or less
63	Phleum spp.	No	High	1	–
64	Pisum sativum	Coarse	High	1	To 17 % moisture content or less
65	Poa spp.	No	High	1	–
66	Raphanus sativus	No	Low	–	–
67	Ricinus communis	Cut	Low	–	To 17 % moisture content or less
68	Secale cereale	Fine	High	2	To 17 % moisture content or less
69	Sesamum indicum	No	Low	–	–
70	Setaria spp.	No	High	1	–
71	Sinapis spp.	No	Low	–	–
72	Solanum lycopersicur	No	High	1	–
73	Solanum melongena	No	Low	–	–
74	Sorghum spp.	Fine	High	2	To 17 % moisture content or less
75	Spinacia oleracea	No	High	1	–
76	Trifolium spp.	No	High	1	–
77	Triticum spp.	Fine	High	2	To 17 % moisture content or less
78	×Triticosecale	Fine	High	2	To 17 % moisture content or less
79	Urochloa spp.	No	High	1	–
80	Valerianellalocusta	No	High	1	–
81	Vicia spp.	Coarse	High	1	To 17 % moisture content or less
82	Vigna spp.	Coarse	High	1	To 17 % moisture content or less
83	Zea mays	Fine	High	4	To 17 % moisture content or less

Reporting of results

Seed moisture content be reported to the nearest 0.1% on ISTA analysis certificate. If the seed moisture content is determined using any moisture meter, the brand name and type of the equipment be mention on the analysis certificate, under column of "other determinations" reporting of range for which the moisture meter is calibrated is another requirement, on seed analysis certificate.

Under "Other determinations," the following extra details must also be included:

- If seeds that were germination stage were found in the sample, the following declaration needs to be made: 'Germinating seeds were found in the submitted moisture sample'.
- The declaration below must be made if mouldy seeds were found in the sample: 'Mouldy seeds were found in the submitted moisture sample'.
- For pelleted seeds, the following declaration needs to be made: 'The seeds of the submitted moisture sample were pelleted, and the moisture content reported is the average of seed and pelleting materials.
- For Arachishypogaea, one of the following statements must be entered: 'The submitted sample for moisture determination consisted of seeds in their pod' or 'the submitted sample for moisture determination consisted of seeds with the pod removed (shelled seeds)'.

References:

Agarwal, R. L. (2018). Seed Technology. India: Oxford & IBH Publishing Company Pvt. Limited.

Khare, D., Bhale, M. S. (2018). Seed Technology. India: Scientific Publishers (India).

J. S. C. Smith and J. C. Register III (1998). Genetic purity and testing technologies for seed quality: a company perspective. Seed Science Research, 8, pp 285-294.

International Rules for Seed Testing, Full Issue i–19-8 (300)

https://seednet.ap.nic.in/Stl/htmlpages/seedmoisturetesting.htm

******

Bt expression analysis -Qualitative and Quantitative

G. Balasubramani
Principal Scientist & Head,
ICAR- Central Institute for Cotton Research

Introduction:

Insecticidal crystal (Cry) proteins of Bacillus thuringiensis (Bt) are entomopathogenic lectins. These proteins act by specifically binding to membrane receptors in the insect midgut, and cause mortality through pore formation and subsequent sepsis. Bacterial Cry protoxins have been used for plant protection purposes in spray formulations for several decades. Characteristic features in the mode of action are: (1) Cry toxin proteins from various Bt strains show insect specificity at family level and (2) bacterial Cry toxin proteins (protoxins) require enzymatic cleavage to generate their active form (activated toxin), mostly by trypsinlike insect gut enzymes. In recent decades, with the advances in molecular biology and genetic engineering, it became possible to identify, isolate and transfer the genes encoding for the production of Cry proteins from the bacillus to plants. A number of plants have been genetically modified (GM) especially cotton (Bt cotton) with full-length natural pro-toxin and truncated version. The Enzyme-Linked Immuno Sorbent Assay (ELISA) is a biochemical technique used mainly to detect the presence of an antibody or an antigen in a sample using two antibodies. ELISA has been widely used as a qualitative and quantitative method for detection of Cry proteins in Bt cotton.

ELISA.

In 1971, two Swedish scientists, Eva Engvall and Peter Perlman, invented a test called the ELISA test that revolutionized medicine; the method uses antibodies to find out the presence of proteins, hormones or viruses etc. ELISA principles is similar to immunoassay technologies, which offer colorimetric/chromogenic substrate for ELISA development with alkaline phosphatase (AP) and three substrates for horseradish peroxidase enzyme (HRP): PNPP (p-Nitrophenyl Phosphate, Disodium Salt) is a widely used substrate for detecting alkaline phosphatase in ELISA applications.

Types of ELISA:

There are four types ELISA tests such as direct ELISA, indirect ELISA, Sandwich ELISA and competitive ELISA. Direct ELISA test which is considered to be the simplest type of ELISA, where the antigen is adsorbed to a plastic plate, then an excess of another protein (normally bovine serum albumin) is added to block all the other binding sites. While an enzyme is linked to an antibody in a separate reaction, the enzyme-antibody complex is applied to adsorb to the antigen. After excess enzyme-antibody complex is washed off, enzyme-antibody bound to antigen is left. By adding in the enzyme's substrate, the enzyme is detected illustrating the signal of the antigen.Direct ELISA is faster because only one antibody is being used and fewer steps are required. This can be used to test specific antibody-to-antigen reactions, and helps to eliminate cross-reactivity between other antibodies. Indirect ELISA is a two-step ELISA which involves two binding process of

primary antibody and labelled secondary antibody. The primary antibody is incubated with the antigen followed by the incubation with the secondary antibody. However, this may lead to nonspecific signals because of cross-reaction that the secondary antibody may bring about. Micro-well plates are incubated with antigens, washed up and blocked with BSA. Samples with antibodies are added and washed. Enzyme linked secondary antibody are added and washed. A substrate is added, and enzymes on the antibody elicit a chromogenic or fluorescent signal.

Sandwich ELISA

Sandwich ELISA is a less common variant of ELISA, but is highly efficient in sample antigen detection. Many commercial ELISA pair sets are built on this sandwich ELISA.The sandwich ELISA quantifies antigens between two layers of antibodies (i.e. capture and detection antibody). The antigen to be measured must contain at least two antigenic epitope capable of binding to antibody, since at least two antibodies act in the sandwich. Either monoclonal or polyclonal antibodies can be used as the capture and detection antibodies in Sandwich ELISA systems. Monoclonal antibodies recognize a single epitope that allows fine detection and quantification of small differences in antigen. A polyclonal is often used as the capture antibody to pull down as much of the antigen as possible. The advantage of Sandwich ELISA is that the sample does not have to be purified before analysis, and the assay can be very sensitive (up to 2 to 5 times more sensitive than direct or indirect ELISA).

The principle behind is plates are first coated with a capture antibody, then the sample is added and any antigen present binds to capture antibody. Later detecting antibody is added, and binds to antigen. Enzyme-linked secondary antibody is added, and binds to detecting antibody and finally substrate is added, and is converted by enzyme to detectable form.

Competitive ELISA principle

The central event of competitive ELISA is a competitive binding process executed by original antigen (sample antigen) and add-in antigen. The procedures of competitive ELISA are different in some respects compared with Indirect ELISA, Sandwich ELISA and Direct ELISA. A simplified procedure is primary antibody (unlabeled) is incubated with sample antigen. Antibody-antigen complexes are then added to 96-well plates which are pre-coated with the same antigen. Unbound antibody is removed by washing the plate. (The more antigen in the sample, the less antibody will be able to bind to the antigen in the well, hence "competition."). The secondary antibody that is specific to the primary antibody and conjugated with an enzyme is added. A substrate is added, and remaining enzymes elicit a chromogenic or fluorescent signal.

The ELISA assay yields three different types of data output:

Quantitative: ELISA test results can be interpreted in comparison to a standard curve (a serial dilution of a known, purified antigen) in order to precisely calculates the

concentrations of antigen in various samples. The samples can be used as fresh or dry weight (lyophilized) basis and expressed microgram per gram of tissues.

Qualitative: ELISAs test can also be used to achieve a yes or no answer indicating whether a particular antigen is present in a sample, as compared to a blank well containing no antigen or an unrelated control antigen. For instanceBt cotton fresh leaf/square/flowers/bolls can be used as test to find out Bt or Non-Bt using qualitative ELISA test.

Semi-Quantitative: ELISAs can be used to compare the relative levels of antigen in assay samples, since the intensity of signal will vary directly with antigen concentration.

The practical aspects of ELISA:
Collection of cotton plant samples from the field and things to be carried for sampling:

- Insulated Thermocol Box with ice pack
- Zip Lock Bags (10x15cm) – Pre labelled
- Big Zip Lock Bags (15x20cm)
- Scissors
- Gloves
- Marker pens
- Coloured Tags (to tag plants)
- Rubber Bands
- Cello Tape (Transparent)
- Field Note book
- Trial Layout

Sampling method at Bt- Cotton field:

1. **Selection of plants:** Select randomly healthy plants from the field. Tag the plants with coloured tags.

2. **Sampling:** Wear gloves, select fully opened 2-3 leaves per plant (normally 3rd leaves from terminal) / 2-squares per plant / 2-bolls per plant and cut with scissor. Put the leaves into a single zip-lock bag which is already labelled or label it. Remove the air from the bags and zip-lock them. Follow the same procedure separately for collection of squares and bolls samples.

3. **Sampling** should be carried out early in the morning at least before 10:00 AM as far as possible, also don't carry out sampling when it's raining, because leaf sample should be somewhat dry to avoid any contamination.

4. **Placement on ice:** Keep the bags containing the samples in the ice pack in the thermocol box in a big zip-lock bag.

5. **Lyophilisation**: Bring to the laboratory and store at minus 20°C deep freezer or subjected to lyophilisation of sample.

Qualitative test of Bt and HT Cotton:

Dip-stick methods are used for qualitative test, which is a simple immunological test that can be carried out in the field condition and even a layman for instantaneous detection of Bt toxin in either seeds or plant tissue. ICAR-CICR has developed Bt-Express kit to detect Bt cry1Ac and other company makes are also available in the market to detect Bt Cry1Ac, Cry2Ab, HT etc. The test can be used in fields and does not require any additional facilities for use and it takes about 5-10 minutes for the test to be completed.

Quantitative test of Bt Cotton:

For precise quantitative estimation of Bt cry proteins expressed in transgenic plants are tested using 96 well ELISA plate. A variety of quantitative ELISA plates are available in the market including BT Quant (developed by ICAR-CICR). The kit is simple, effective and very reliable but it needequipments especially ELISA reader and other lab facility to carry out. It takes about four hrs for completion of one set of ELISA assay and each plate can be used for 96 samples per test.

For qualitative and quantitative analysis DesiGen kit / protocol are being used at ICAR-CICR, which has been given in the Annexure-1 (for Cry1Ac for qualitative) and Annexure-2 (for Cry2Ab for quantitative assay) for reference.

Annexure-1

Qualitative assay for Cry 1 Ac (DesiGen) 96-well ELISA plate:
Assay Time: 60 min

Intended use:
The Cry1Ac plate is design for qualitative (presence/ absence) laboratory detection of cry1Ac protein in cotton seed or cotton leaf tissue samples.

Storage:
The plate should be stored at 4° C- 8° C and the components should be stored as mentioned on the respective bottles/ vials to get maximum shelf life. Do not expose to temperature in excess of 37° C. Avoid direct sunlight.

How the plate works:
Seed or leaf extracts are added to wells, pre-coated with anti-Cry Ac antibodies. A secondary Anti- Cry Ac Antibody Conjugate is added to the well prior to plant samples. After a wash, substrate is added to detect Cry 1 Ac protein in samples via a colour reaction.

Material provided:
Cry1 Ac mAb Coated ELISA Plate
Anti- Cry Ac conjugates
Cry 1 Ac positive Control
Negative control
10 X Extraction Buffer (Premix)*
10 X WASH Buffer (Premix)*
Ready to use TMB Subtract (HRP)
Stop Solution

RECIPE:

a) *** 10 X Extraction Buffer** (500 ml)
   Mix the 10 X Extraction Buffer (Premix) in 400 ml ofMill-Q /Distilled water. Add 2.5 ml of Solution- A per 500 ml (provided in viral) and mix well. Make up the volume to 500 ml using Mill-Q /Distilled water.
b) **1 X Extraction Buffer:**
   Take 50 ml of 10 X Extraction Buffer, dilute it to 500 ml by adding D/ W. Use 500 µl or 1 ml. per sample.
c) ***10 X WASH Buffer (500 ml):**
   Mix the Wash Buffer (Premix) in 400 ml ofMill-Q /Distilled water. Add 5 ml of Solution- A per 500 ml (provided in viral) and mix well. Check the pH (pH should be 8.5-9.0). Make up the volume to 500 ml using Mill-Q /Distilled water.
d) **1X WASH Buffer (wash Buffer):**
   Take 100 ml of 10 X Wash Buffer; dilute it to 1 L by adding D/W.

Criteria for a valid ELISA test/ classification of positives:
Sample well with all reagents added except seed/ leaf extract (blank wells), should have an absorbance value below 0.200 at 450 nm at the time of taking the reading. A positive sample is indicated by a value above cut off value, after zeroing the instrument to a blank well.

Sample preparation:
Note: Avoid cross – contamination between samples
For seed extracts: Imbibe cotton seeds overnight in water. Remove seed coat and place the seed in a micro-centrifuge tube and add 500 µl or 1 ml. 1X Extraction buffer (Refer to recipe). Crush with a pestle for 30 seconds. Use 50 µl of this extract for loading.

For Positive and Negative Control: Take 20-30 mg (Approx) of control powder, add 500 µl or 1 ml. 1X Extraction buffer. Crush with a pestle for 30 seconds. Use 50 µl of this extract and remaining can be used for a week, when stored at -20^{o} C or -80 ^{o} C.

For leaf extracts: Punch out 3-4leaf disc with a mcf tube by placing a leaf between the lid and tube opening and closing the lid onto the leaf. Add 500 µl 1X Extraction buffer (Refer to recipe). Crush with a pestle for 30 seconds. Use 50 µl of this extract for loading.

Assay:

1. To the Cry1 Ac mAb Coated ELISA Plate, add 50 µl / well of Anti –Cry1Ac conjugate.
2. Add 50 µl of the sample extract, positive control and negative control provided to the appropriate wells. Add 50 µl 1X Extraction buffer in blank well. Incubate the plate at room temperature for 45 min.
3. Wash the plates four times with1X (Wash Buffer) Pat the plates dry (wells down) on blotting paper to remove excess buffer.
4. Add 100 µl of Ready to use TMB Substrate (HRP) per well (Take care not to touch the wells while adding the subtract)
5. Incubate the plate at room temperature in dark for 15 min. Add 100 µl/ well stop solution after 15 min of incubation.
6. Measure the absorbance at 450 nm using an ELISA plate reader. The absorbance of blank well must be subtracted from absorbance values of samples and controls.

Assay Acceptance Criteria and Interpretation:
Blank: The OD value of blank well should be below 0.200
Negatives: The OD value of negative control should be below 0.100
Positives: Samples showing absorbance above cut off value.
Note: First subtract OD value of blank form all readings and if negative control shows in minus readings then consider that reading as zero for cut off calculation.

- **Cut off value= (O.D. of negative control) + 0.2**

Annexure-2

ELISA 96-well plate kit for quantitative estimation of Cry2Ab protein.
(DesiGen Kit protocol)

The Cry2Ab plate is designed for quantitative laboratory detection of Cry2Ab protein in cotton leaf, square and boll tissue samples.

Storage and validity:
The plate should be stored at 4- 8° C. Do not expose to temperatures in excess of 37^0C. Avoid direct sunlight. When stored properly, the plate is valid for 6 months.

How the plate works:
Tissue extracts are added to wells coated with antibodies raised against Cry2Ab. Cry2Ab residues in the plant sample bind to the antibodies. A secondary antibody against Cry2Ab is added to the wells, followed by plant samples. After a wash step, a third antibody, labelled with an enzyme (alkaline phosphatase) is used to detect Cry2Ab protein in the sample via a colour reaction.

Materials provided in the kit:

1) Cry2Ab mAb Coated ELISA Plate
2) Ab2 For Cry2Ab
3) Tertiary Antibody (Ab3)
4) Cry2Ab protein stock (16ug/ml)
5) QuanT Extraction Buffer
6) Powder A
7) Powder B
8) Ovalbumin
9) 10X Buffer A
10) 5X Substrate Buffer
11) pNPP (Substrate)
12) Cry2Ab Positive Control
13)Negative Control

Sample preparation:

For leaf, Square and boll tissues:

- Place 5mg of lyophilized tissue in a 1.5-ml microfuge tube.
- Add 500 µl of ice-cold 1X Extraction buffer (Freshly prepared)
- Mecerate tissue powder at 3000 rpm using a motor driven pestle or 30 sec.
- Chill on ice for 10 min and again macerate for 30 sec.
- Spin at 8000 rpm for 15 min and pipette out the supernatant.

Dilute the supernatant appropriately as given under **Plate loading**.

Preparation of positive and negative QC seed extracts:
Note: These samples are for qualitative evaluation of the plate.

1. Add 500 µl of 1x Buffer A to the positive and negative seed samples provided with the kit. Crush well with a disposable plastic pestle. Spin for 30 sec in a

microcentrifuge. **Dilute Positive seed extract 1:20 in 1X Diluent Buffer** and use 100ul of each supernatant per well.

2. Extracts can be stored in 100 µl aliquots and frozen at -80^{0}C and used for up to 3 months.

Standard curve generation:

Prepare a 160 ng/ml working stock solution from the 16ug/ml Cry2Ab stock solution provided, in **1X Diluent buffer** (1:100 dilution)

Generate the other quantification standards as follows:

Std. No	Quantification standards scheme	Sample buffer (ul)	Cry2Ab conc. (ng/ml)
1	500 ul of 160 ng/ml Cry2Ab soln.	500	80
2	500 ul of std.1	500	40
3	500 ul of std.2	500	20
4	500 ul of std.3	500	10
5	500 ul of std.4	500	5
6	500 ul of std.5	500	2.5

Goat anti-Cry2Ab (Ab2) Preparation:

- Dilute Goat anti-Cry2Ab (**Ab2)** 1:1000 in **1X diluent buffer**.
- Add 150ul of Ab2 to each well.

Plate loading:

Add 100 ul of the buffer blank, standards, Positive and negative QC samples and samples diluted in **1X diluents buffer** as indicated below. This is the "Most concentrated" dilution that the samples may be loaded at. Samples may need to be loaded at a more dilute dilution.

Tissue	Sample buffer	Dilution
Leaf	1X diluents buffer	1:80
Square	1X diluents buffer	1:100
Boll	1X diluents buffer	1:50

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blnk	Std1	Std1	Std1	S3	S5	S8	S11	S13	S16	S19	S21
B	Blnk	Std2	Std2	Std2	S3	S6	S8	S11	S14	S16	S19	S22
C	+ve	Std3	Std3	Std3	S3	S6	S9	S11	S14	S17	S19	S22
D	+ve	Std4	Std4	Std4	S4	S6	S9	S12	S14	S17	S20	S22
E	+ve	Std5	Std5	Std5	S4	S7	S9	S12	S15	S17	S20	S23
F	-ve	Std6	Std6	Std6	S4	S7	S10	S12	S15	S18	S20	S23
G	-ve	S1	S1	S2	S5	S7	S10	S13	S15	S18	S21	S23
H	-ve	S1	S2	S2	S5	S8	S10	S13	S16	S18	S21	Blnk

- Incubate the plate at 37^0c for 1.5 hour in a humid environment.
- Discard samples and wash the plate with **1X wash buffer** twice, allowing the plate to stand 5 minutes with wash buffer in the wells between washes.
- Patting the plate dry on paper towels.

Conjugate preparation:

- Dilute AP-Conjugated **Ab3** 1:1000 in **1X diluents buffer**.
- Add 250 ul per well. Mix and incubate for at 37^0C for 45 min in a humid environment.
- Wash the plate with **1X wash buffer** twice, allowing the plate to stand 5 minutes with wash buffer in the wells between washes. Pat the plate dry on paper towels.

Substrate preparation:

- Add 250 ul per well of a 1 mg/ml pNPP solution prepared in freshly prepared **1X substrate buffer.**

Development:

- Incubate at room temperature in the dark for 30-45 min.

Data generation:

- Read the absorbance of the plate at 405 nm after setting one of the Blank wells as a blank.
- **Use Sigma Plot^{R}version 8.01 (4-Parameter sigmoidal curve fit) to generate a standard curve**.
- Determine the Cry2Ab concentration of each sample by finding its OD value (average of triplicate samples) and the corresponding concentration level from the software generated plot.

Dry weight expression level:
To calculate µg/g dry weight levels for all tissues, use the following
µg/g dwt= (ng/ml value from Sigma Plot^R software) (Dilution factor) (T: B ratio)/1000

RECIPES:

a) 1X Extraction Buffer (100 ml)

Add 0.2 g powder A and 12 g powder B to Extraction buffer provided with the kit, freshly at the time of the sample extraction. This buffer should be used within **4 hours** of preparation. After extraction, samples can be used immediately or can be stored at -80^0C for one day.

b) 1X wash buffer

Take 100 ml of **10X Buffer A**, dilute it to 1L by adding deionized water.

c) 1X Diluent buffer:

Take 100 ml of **10X Buffer A**, dilute it to 1L by adding deionized water. Add 0.5% Ovalbumin in 1X Diluent buffer. Store the solution at 4^0C.

d) 1X Substrate buffer:

Add 20 ml of deionized water to 5ml of 5X –substrate buffer provided (This can be stored in a dark bottle in the refrigerator for one week).

e) pNPP substrate:

Add 25mg of pNPP to 25ml of 1X substrate buffer (prepare this substrate 10 min before adding and keep it in the dark).

*****

Seed Varietal Purity Testing through DNA Fingerprinting

J.Amudha.
Principal Scientist,
ICAR- Central Institute for Cotton Research, Nagpur

Introduction

The DNA fingerprinting is the technique of finding the genetic identity. This is primarily based on the polymorphisms occurring at the molecular level that is on the base sequences of the genome. The fundamental techniques involved in genetic fingerprinting were discovered serendipitously in 1984 by geneticist Alec J. Jeffreys of the University of Leicester in Great Britain while he was studying the gene for myoglobin, a protein that stores oxygen in muscle cells. The technique crossed the arena of the scientific frontiers mainly with the application in the forensics. With advent of time, development of various techniques paved way for the use of this technique in different fields giving newer dimensions to this Technique. The DNA profiling is primarily used in plants for protection of biodiversity, identifying markers for traits,identification of gene diversity and variation etc. The most popular or widely used techniques used with relevant to plants are **RFLP, RAPD, ISSR, SSR** etc.

Techniques used in DNA Fingerprinting

What are molecular markers: Molecular markers are stable changes (often affecting a single base) in short DNA sequence that occurs at specific locations on the chromosomes. Different methods have been developed to identify these changes. When individual have different sequence in the DNA at a particular chromosome region, such that the scientists can distinguish among the individuals, the molecular marker is said to be polymorphic. Molecular markers usually follow the same rules of inheritance as genes.

Types of molecular markers: Molecular makers can be grouped into three broad classes with respect to basic strategy employed:

- Non-PCR based molecular markers
- PCR based molecular markers
- Targeted PCR and sequencing

Polymerase Chain Reaction (PCR):

The DNA amplification by thermal cycling called Polymerase Chain Reaction is in vitro method that can be used to amplify a specific DNA segment from small amounts of DNA template or duplex into millions of copies. It is invented by Kary Mullis *et.al,* (1985).

Steps involved in PCR are:

Heat Denaturation.
Annealing.
Primer Extension.

Heat denaturation:

This temperature denatures the double stranded DNA into two individual stands. Denaturation temperature is 95^0 C for 30 seconds or is 97^0 C for 15 seconds, however higher temperature may be appropriate, especially for G+C rich nucleotides.

Annealing:

During this time one primer binds with the 5 prime end of one DNA strand and the other primer binds with 3 prime end of its complementary strand .Annealing is hybridization of primers to single stranded DNA and the length of time required for primer annealing depends on the basic composition, length and concentration of primers.

Primer Extension:

This temperature is varies for Taq DNA polymerase which adds complementary nucleotides one by one to the 3' OH group of the primer. Estimates for the rate of nucleotide incorporation at 72^0 C vary from 35-100 nucleotides per second depending upon the buffer, pH salt concentration and nature of DNA template.

Number of PCR Cycles:

The optimum number of cycles depends mainly upon the starting concentration of target DNA, when other parameters are optimized. A common mistake is to execute too many cycles. The increase in the number of cycles will increase the amount and complexity of non-specific background products.

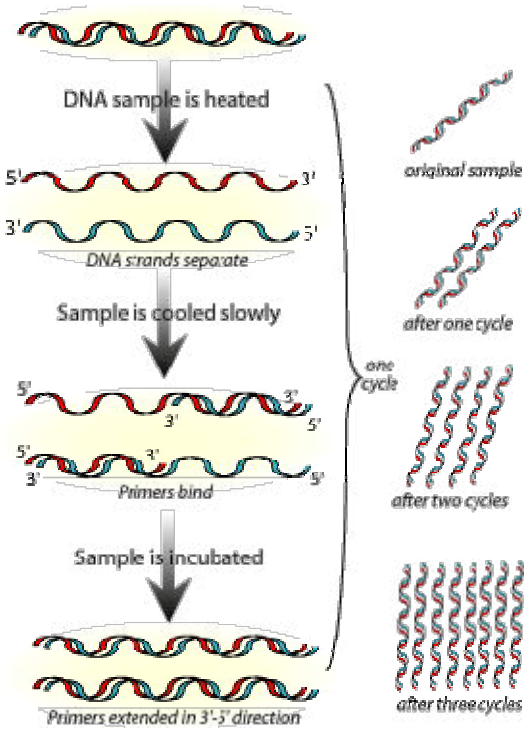


Figure 1.The Polymerase Chain Reaction is used to amplify a sample of DNA.

1. **Microsatellites are simple sequence repeats (SSRs)**, 1 to 6 nucleotides in length, which show a high degree of polymorphism. Specific microsatellites can be isolated using hybridized probes followed by their sequencing. Like any DNA fragment, SSRs can be

detected by specific dyes or by radiolabelling using gel electrophoresis. The advantage of using SSRs as molecular markers is the extent of polymorphism shown, which enables the detection of differences at multiple loci between strains [3].Coupled with chemical and morphological data, we can identify the plant species or strain of interest. The main advantage of using SSRs for fingerprinting is that small amounts of DNA are required compared to the restriction fragment length polymorphisms (RFLP) method. This is due to the large amounts of SSRs present in any genome. Further, assays involving SSRs are more robust than random amplified polymorphic DNA (RAPDs), making them up to seven times more efficient. A drawback to using SSRs is the need to develop separate SSR primer sets for each species. The latest research suggests that SSRs will be involved in new methods of detection of alterations of specific sequences in the DNA.

2. **Restriction fragment length polymorphisms** are unequal lengths of DNA fragments obtained by cutting Variable Number of Tandem Repeat (VNTRs) sequences up to 30 sequences long with restriction enzymes at specific sites. VNTRs vary between plant species, as do the number and location of restriction enzyme-recognition sites. On an agarose gel, RFLPs can be visualized using radiolabeled complementary DNA sequences. There is no need for PCR amplification of DNA in this method. A routine southern blot experiment is used instead. Normally, RFLPs are used to identify the origins of a particular plant species, setting the stage for mapping its evolution. There are some problems with the RFLP method of DNA fingerprinting. First, the results do not specifically indicate the chance of a match between two organisms. Secondly, the process involves a lot of money and labor, which not many laboratories can afford. Finally, unlike the microsatellites, a few loci in the assay must suffice.

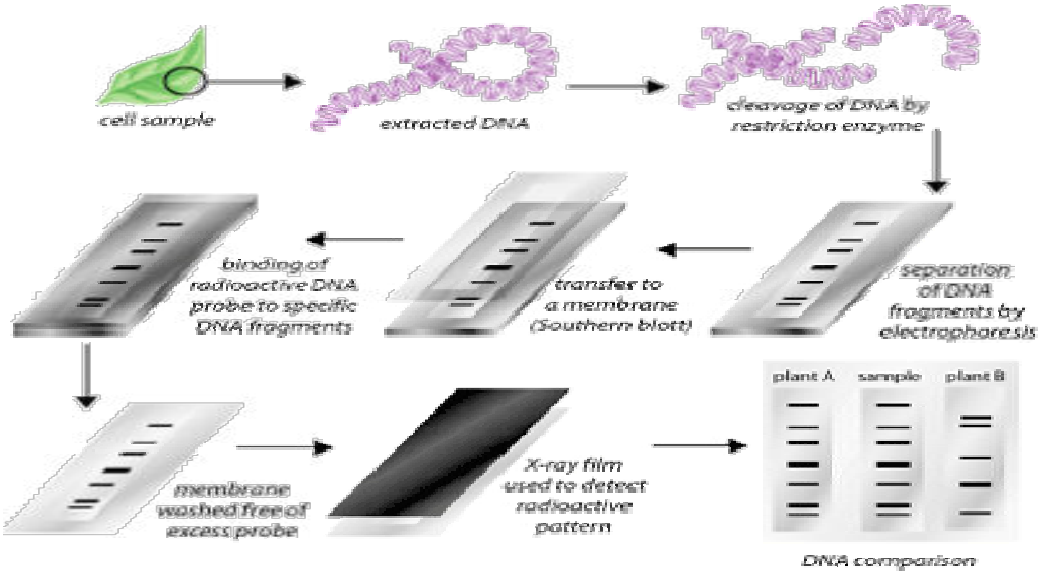


Figure 2. RFLP is one of the DNA fingerprinting techniques that is used to determine plant variety and purity

3. **Amplified fragment length polymorphism (AFLP)** is a PCR-based derivative method of RFLP in which sequences are selectively amplified using primers. It is a reliable and efficient method of detecting molecular markers. DNA is cut with two restriction enzymes to generate specific sequences, which are then amplified suitably. The mere addition or

deletion of bases at the 3′ end determines the selectivity and complexity of the amplification 4. By using AFLP, it is possible to evaluate more loci than with RFLP or RAPD. AFLP is also capable of determining a large number of polymorphisms. Similar to SSRs, AFLP-based assays are cost-effective and can be automated.

4. **Random amplified polymorphic DNA** is one of the most commonly used primary assays for screening the differences in DNA sequences of two species of plants. RAPD consists of fishing for the sequence using random amplification. Here, plant genomic DNA is cut and amplified using short single primers at low annealing temperatures, resulting in amplification at multiple loci. By running a 2-dimensional electrophoresis gel, it is possible to determine the change in sequence pattern by superimposing the 2 gels. Once the band of interest is identified, the gel is cut, and the DNA is isolated and sequenced. Using this target, DNA from other *cultivars* can be assessed using other techniques such as AFLP or SSRs. It is also more cost effective than RFLPs. RAPDs lack specificity, however, due to low annealing temperatures and easier reaction conditions.
5. Other Methods include the use of **single nucleotide polymorphs (SNPs),** DNA amplification fingerprinting (DAF) and their offshoots. Although these techniques vary slightly from each other, they operate on the same principle.

6. **Arbitrarily Primed Polymerase Chain Reaction (AP-PCR)** is a special case of RAPD developed by Welsh and McClelland, 1990, wherein discrete amplification patterns are generated by employing single primers of 10-50 bases in length in PCR of genomic DNA. In the first two cycles annealing is under non-stringent conditions. The final products are similar to RAPD products. Recently, separating the fragments on agarose gels and using ethidium bromide staining for visualization has simplified this technique.

7. **DNA Amplification Fingerprinting (DAF)**: Caetano-Anolles*et al.*, 1991 employed single arbitrary primers as short as five bases to amplify DNA using PCR. In a spectrum of products obtained, simple patterns are useful as genetic markers for mapping, while more complex patterns are useful for DNA fingerprinting. Products of DAF are analyzed on acrylamide gels and detected by silver staining. DAF requires careful optimization of parameters; however it is extremely amenable to automation and fluorescent tagging of primers for early and easy determination of amplified products.

8. **Cleaved Amplified Polymorphic Sequences (CAPs)** are generated by restriction enzyme digestion of PCR products. Such digests are compared for their differential migration during electrophoresis. These are co dominant in nature.

9. **Diversity Arrays Technology (DArT array)** method is hybridization of high throughput genotyping. This is a microarray-based DNA marker technique for genome-wide discovery and genotyping of genetic variation. DArT allows simultaneous scoring of hundreds of restriction site based polymorphisms between genotypes and doesn't require DNA sequence information or site-specific oligonucleotides.

Isolation of plant DNA

Nucleic acids (DNA and RNA) are vital micro-molecules in all living cells. DNA contains the basic genetic information of an organism. In prokaryotic cells, genetic activity occurs throughout the cell while in eukaryotic cells it lies in the discrete particles within the cells. Most of the DNA of eukaryotes exists in the nuclei and remaining DNA in the partially self-duplicating mitochondrial and chloroplast particles. The nuclear DNA combines with histone proteins in an orderly manner to form chromatin.

Extraction of DNA is done by number of methods. The efficiency and recovery of extraction depends on the sample material, ionic conditions of the extraction medium and type of lysing agent used, etc.

- Extraction of DNA is accomplished by the rupturing of cell wall and nuclear membrane followed by deproteinization and precipitation of nucleic acid using ethanol. A number of methods are available for the extraction of plant DNA depending upon the starting tissue, homogenization conditions, etc. in the present investigation we are following the isolation method developed by Paterson *et al.,*(1994) .

Protocol used for isolation of DNA from Cotton

Materials

- Sample material (Leaf or Seed from cotton)
- Extraction buffer (pH 7.8)
  - 100mM Tris-HCl - 1.576 g
  - 10mM EDTA Na_2 - 0.372 g
  - 500mM NaCl - 2.992 g
  - Distilled Water - 100 ml
- Suspension Buffer (pH-8)
  - 50mM Tris-HCl - 0.788 g
  - 10mM EDTA Na_2 – 0.372g
  - Distilled Water - 100 ml
- 7.5M Ammonium acetate -57.81g in 100ml
- 20% SDS (Sodium Dodecyl Sulphate)
- 20% PVP (Poly Vinyl Pyrrolidone)
- 80% Ethanol
- TE Buffer (pH-7.5)
  - 10mM Tris-HCl - 0.157 g
  - 1mM EDTA Na_2 - 0.37 g
  - Distilled Water - 100 ml

Method:

- Weigh 0.1 g of plant tissue, quickly freeze in liquid nitrogen and grind to fine powder in a pestle mortar.
- Add 2 ml of extraction buffer in small aliquots and grind thoroughly.
- Add 1 ml of suspension buffer and make the suspension.
- Transfer the homogenate to a centrifuge tube and add 250 µl of 20% SDS and 20% PVP. Mix the contents properly.

- Keep the tube in water bath at 65˚C for 30min with inversion at every 10min. Take out the tube and add 3ml of 7.5M ammonium acetate and keep in ice for 1hr.
- Centrifuge the contents at 15000rpm at 4˚C for 15min.
- To the aqueous layer add 6/10th volume of isopropyl alcohol and keep at -20˚C overnight for complete precipitation of DNA.
- Pellet the DNA by spinning at 15000rpm for 15min. dissolve the pellet in 25µl of TE buffer add 1.5µl RNase for 15 min at 37˚C.
- Pellet the DNA by centrifugation at 1500rpm for 15min. if DNA is not pure, go for phenol:chloroform and isoamyl alcohol mixture. Mix properly by inverting the tube 4 to 5 times.
- Centrifuge and pull out the aqueous layer in a fresh tube leaving the interphase.
- Add equal volume of ice-cold ethanol and pellet the DNA by centrifugation at 15000rpm for 15min (repeat this step at least 2 times).
- Drain off the ethanol and invert the tubes on tissue paper towel for 1min. allow the pellet to air dry, take care that not to dry the pellet to much.
- Dissolve the pellet in suitable volume (30µl) or TE buffer.

Quantification of DNA

The isolated DNA was quantified in Spectrophotometer at 260nm.Concentration of 50µg/ml shows absorbance of 1.0 at 260nm. The 260/280 ratio should be 1.8-2.0 for good quality DNA.

Agarose Gel Electrophoresis

Preparation of Agarose Gel

For checking the quality of DNA, 0.8% agarose was used. For electrophoresis of PCR amplified DNA products 1.5% agarose gel was used.

Materials:

- 10X Tris-Borate EDTA (TBE) buffer (pH 8.0)
  - Tris buffer - 108.0 g
  - Boric acid - 55.0 g
  - 0.5M EDTA (pH 8.0) - 2.925 g
  - Distilled water - 1000 ml

  The solution was prepared and autoclaved.
- Gel Loading Dye
  - Sucrose - 66.7 mg
  - Bromophenol blue - 4.2 mg
  - Distilled water - 1.0 ml
- Gel electrophoresis unit and power pack (Bangalore Genei Pvt. Ltd.).

Method:

- The ends of the gel casting plate were sealed with cello-tape and kept on perfectly horizontal leveled platform.
- Agarose was melted in 1X TBE buffer and allowed to cool lukewarm temperature and then ethidium bromide (10 mg/ml) was added at final concentration of 1 µl per 25 ml of agarose gel.
- The agarose was poured into the gel casting plate with the comb fixed on one end of plate and the gel was allowed to solidify.
- After solidification of the gel, the comb was carefully removed from the gel plate without disturbing the wells.
- The gel cast was now placed on the electrophoresis unit and submerged with 1X TBE buffer.
- The DNA samples were mixed with the gel loading dye and carefully loaded into the wells. A suitable DNA ladder was also loaded.
- The cathode and anode were connected to the power pack and the current was adjusted to 80V.
- The negatively charged DNA molecules moved towards the anode and they separated according to their molecular weight. The power was turned off when the tracking dye reached at about 2 cm from the anode end.

Viewing the Gel

After disconnecting the power supply, the gel was transferred on UV-transilluminator and viewed. DNA in the gel appeared pinkish-orange owing to fluorescence of bound ethidium bromide (intercalating chemical). The gel was photographed and was used for further interpretation of band patterns.

DNA Amplification by PCR

Polymerase chain reaction is an ingenious new tool for molecular biology. PCR is very sensitive method that a single DNA molecule has been amplified and single copy genes are routinely extracted out of complex mixtures of genomic sequences and visualized as distinct bands on agarose gels.Polymerase is an enzyme that polymerizes any DNA material *in vitro*, if it is supplemented with other minimal requirements such as dNTPs (deoxy nucleotide triposphates) and the primers.

The cocktail is incubated at different temperatures e.g. 94°C, 45°C and 72°C which are responsible for the denaturation of the DNA, annealing temperature of primers with the DNA and polymerization of nucleotides on template DNA strand respectively. The PCR protocol differs with different situations.

PCR Principle:

The core of the gradient PCR (Biometra®) is the high performance thermo block high power Peltier elements temper a sample block made of gold plated solid silver. This achieves high heating and cooling rates. Also there is a facility of setting a temperature

gradient to carry out the priming reaction at different temperatures; here a range of temperature is entered in to the instrument e.g. 55°C-65°C, accordingly the instruments sets 12 different temperatures in 12 different wells in a row of sample block.

Materials:

- DNA sample
- 25mM dNTPs
- 25mM MgCl2
- 10 X PCR buffer (Taq buffer B)
- Primer
- Sterile distilled water
- Taq DNA polymerase

PCR protocol

Method:

- A master mix (reaction mixture) was first prepared in one eppendorff tube as follows,For one sample (reaction mixture):

Sr. No.	Reagents	Quantity
1	Sterile distilled water	9.5µl
2	Taq buffer B	2µl
3	MgCl2	3µl
4	dNTPs	2µl
5	Primer (forward)	1µl
6	Primer (reverse)	1µl
7	Taq polymerase	1µl
8	DNA template	1µl
Total		**20.5µl**

- The master mixture was mixed properly and 19µl of it was distributed to each tube in which then 1µl of template DNA was added.
- The tubes was placed in the PCR machine, and the gradient was set having temperature range 55°C-65°C with following PCR program:

  Step 1: Temp-94°C, Time-5min
  Step 2: Temp-94°C, Time-45sec
  Step 3: Temp- 56°C Time-45sec
  Step 4: Temp-72°C Time-60sec
  Step 5: **35 cycles**
  Step 6: Temp-72°C, Time-5min
  Step 7: Temp-4°C Time- variable
- After completion of all the cycles, the tubes was taken out and preserved at 4°C.
- The amplified samples were resolved on 1.5% agarose gel and observed the banding pattern under UV-light.

******

Genetic Purity Analysis through Grow Out Test

Nakul Gupta
ICAR-Indian Institute of Vegetable Research, Varanasi 221305, U.P.

Genetic purity of seed is the most important parameter of seed quality as it determines the authenticity of seed and yield potential of variety/hybrid. In general, the negative impact on seed yield, consistency and quality is due to the presence of a large proportion of off-types. In the scientific production of seeds, special attention is paid to every step to maintain the authenticity of the variety."Genetic Purity of a seed lot is determined on the basis of distinct morphological characteristics of the variety expressed at seed, seedling and plant level by comparing its submitted sample with authentic sample under identical environmental condition."

Source of contamination

Generally, all these kind of impurities occurs as result of delusion or inaccuracy during seed multiplication, harvesting, drying, handling or packaging of seed. For example-

- Seed fertilized by foreign pollen, especially in open pollinated crops
- Mutation
- Unclean harvesting equipment
- Carelessness at the processing
- Mistakes in bagging and tagging
- Mechanical mixture of seeds of other varieties during sowing, harvesting or storage
  In some cases, two additional sources of contamination have appeared as a result of the particular method of seed production used in hybrids.
- Incidental collection of male rather female inbred.
- Sibs (seed produced by self or sib-pollination among female lines.

  All of these sources of contamination are preventable through prudent management. Sibs are the main concern in hybrids and they are difficult to avoid therefore it may cause a large proportion of contamination. Sibs originates from the failure of crossing management such as emasculation, male sterile or self-incompatibility.Examination to determine the genesis of the variety can be conveniently divided into the following three groups:
  - Laboratory examinations
  - Tests in glasshouses or growth chambers.
  - Field trial and field inspection.

  In that article, we discussed Grow-Out-Test, which is part of the field testing and inspection.

Grow-Out-Test

The grow out test is a widely used method for testing the genetic purity of seed samples. This test involves planting seeds in a controlled environment and observing the resulting plants for any off-types or impurities. The grow out test is a non-destructive test, meaning that the seeds can be used for planting after the test is complete. This test is relatively easy to perform and can be conducted in a short amount of time. However, the test may not be

reliable in all situations, as environmental factors can affect the growth of the plants.The main purpose of the magnification test is to establish the authenticity (true-to-type) of the variety. The determination is based on the observation of plant characteristics of a variety that are least environmentally influenced and are highly heritable. In general, differences between varieties are most pronounced under favorable growing conditions. In plot trials, the condition must be set up in such a way that the genetic differences to be examined are as clear as possible.

The amount of seed to be used on a plot is calculated on the basis of qualities of the seed. Before planting, seeds easily distinguished in the laboratory to be of another cultivar are separated, and their portion of the seed quantity is calculated. If possible, questionable seeds can also be separated, which can be seeded separately and examined in greater detail.

The different samples of the same cultivar are seeded in adjacent plots, with typical samples at appropriate intervals. This is especially important for cross-fertilized crops, where the examination, for the most part, is based on a comparison between the samples to be tasted and the standard sample.

The field plot must be carefully observed during the growing session, the appearance of each of the same cultivars is compared to the others, and particularly to the standard sample. The consistency of the stand is also considered. It is particularly worth noting when shooting and/or flowering starts, and how long each period lasts.

Sampling:

Submitted sample: The submitted sample for grow out test is drown simultaneously with submitted sample for other test. The sample size will vary depending on the plant species.

Table 1: Recommended sample size for growth test submission:

Crop	Size of sample (g)
Genera with seed size similar to pearl millets	100
Genera with seed size similar to *Beta vulgaris*	250
Sorghum, rice, wheat and other genera of similar seed size	500
Maize, cotton, groundnut, soybean and other of similar seed size	1000

Note:*The amount of the submitted sample is doubled if it's necessary to determine genetic purity at both the seed and plant levels.*

Working Sample: In order to observe the acceptable off-type plants specified as the minimum seed certification standard in the optimal population, i.e., at least 400 plants, the size of the working sample mostly depends on the test weight and germination percentage of the crop.

Table-2: Number of plants require for grow out test

Maximum permissible Off-types (%)	Number of plants required per sample f observation
0.10	4,000
0.20	2,000
0.30	1,350
0.50	800

1.00 and above	400

Planting Instructions:

1. To make sure that an approximately similar number of plants of the same species or cultivar are established in all plots, the weight of the seed sown should be adjusted in the event that the germination of the sample being sown exhibits significant variation.
2. It must be carefully checked to make sure that it doesn't already contain seed from a previous sample before adding another sample to a seed drill.
3. For each sample, there should be a minimum of two replicate plots. a fallback strategy or an alternative region inside the same field.
4. Any realistic size for the plots is acceptable as long as there are sufficient plants present to make the calculation with the required level of precision.
5. If the seed is planted *in situ*, it should ideally be mechanically planted in rows.
6. Plants and rows should be separated from one another sufficiently to allow for the development of the features being studied.
    a. Cereals, legumes and oil plants: Every plot should be seeded with a convenient number of rows. The recommended row spacing for flax and cereals is 200 to 250 mm, whereas the ideal row spacing for the other species listed below is 400 to 500 mm. The following number of plants per meter of row need to be thought of as ideal:

Table-3: Number of plants per meter of row:

Crop	Plants/Meter	Crop	Plants/Meter	Crop	Plants/Meter
Linum	100	*ViciaFaba*	10	*Pisum*	30
Cereals	60	*Other Vicia*	30	*Lupinus*	30
Brassica	30	*Papaver*	50	*Glycine*	30

The specification for different crops given in the Indian minimum seed certification standards are given in the Table-3. The certification agency may, however, change these specification, if deemed necessary.

Table-4: Spacing specifications

S. No	Crop	Row length (meters)	Plant to plai distance (cn	Space betwee rows (cm)	Space between plots (cm)	No. of replications
1.	Wheat, barley, oats	6	2	25	50	2
2.	Pea, cowpea	6	10	45	90	2
3.	Chickpea, green grai black gram	6	10	30	60	2
4.	Maize	10	25	60	90	2
5.	Hybrid cotton	5	10	45	45	2
6.	Paddy:					
	very early to medium	6	15	20	45	2
	late and very late	6	25	30	60	2
7.	Pearl millet	6	10	60	90	2

8.	Sorghum	6	10	45	60	2

a. *Herbage plants:*It is advised to use rows that are between 300 and 450mm apart and measure approximately 15m in length overall.
Where it is possible to discern between two or more cultivars through the examination of single plants, a special plant approach should be applied. Single plants are often grown by sowing each seed separately in a greenhouse or laboratory. The plants are moved onto field plots once they have reached an appropriate size. Under ideal circumstances, it may be feasible to sow the seed in place, in which case seedlings are separated into single plants. Plants should be spaced apart by at least 600mm in both directions.

b. *Root and Other Crops:* Root and other crops grown spaced in rows.Each plot should include at least two rows, with a total length that will provide 400 or more plants for analysis. In order to grow about the same number of plants in the test and control plots, the sowing rate should be modified because both transplanting and thinning are potential sources of error. Only when it is deemed to be absolutely required is it possible to thin out or transplant from another part of the plot.

Recording of observations:

Throughout the entire growth season, observations should be conducted, and any differences from the control sample should be noted. Plants that are easily identifiable as being of a different cultivar, species, or as aberrants should be counted and noted.

1. **Estimating the number of plants**
When possible, the number of plants in the plot should be counted or estimated, ideally while the plants are being studied. This is required in order to give the field plots test's estimated percentage of aberrant individuals.
Evaluation in conjunction with check counting is used for unthinned crops like grains. The plot contains at least two repeating locations where the number of individuals per meter of row is counted. The total number of plants in the plots can be computed using these counts. The best time to do this activity is after the plants have fully emerged but before they begin to tiller.
It is highly impractical to count the number of plants on unthinned plots in perennial, strongly tillered species, such as herbage seeds. In these species, the quantity of aberrant plants may be expressed as a function of area, number of seeds dispersed, or another appropriate metric.
2. **Taking observations**

The minimum number of plants that needs to be examined are given in following table. The minimum number is dependent on maximum permissible off-types.

Table-5: Minimum number of plants to be observed in GOT

Maximum permissible Of types (%)	**Minimum genetic purity (%)**	**Number of plants required p sample for observation**
0.10	99.9	4,000
0.20	99.8	2,000
0.30	99.7	1,350
0.50	99.5	800

1.00 and above	99.0 and below	400

Expression of Results:

a. Seeds and Seedlings: The findings of the determination of the seedlings are provided as a percentage of the number of typical seedlings evaluated.
b. When possible, the number of plants discovered to be of different cultivars, different species, or aberrant shall be calculated as a percentage of the total number of plants tested.

In the case of herbage plants and related species, when cultivated in rows with broad spacing, it is difficult to quantify the total number of plants inspected per plot. The outcome may be stated as the number of divergent plants produced by the weight of seed dispersed.

The mean and other statistics can be calculated when characters are measured. It is frequently difficult to describe properly all off-types in cultivars of cross-fertilizing species, such as rye, root crops, herbage plants, etc. In this situation, any estimations of percentage impurity should be accompanied by relevant comments about the veracity of test samples.

Tolerance may be applied by using the reject table given below.

Table-6 Reject number for prescribed standards and sample size:

Standard	**Reject numbers for sample size of**	
	800	**400**
99.5 (1 in 200)	8	*
99.0 (1 in 100)	16	8
95.0 (5 in 100)	48	24
90.0 (10 in 100)	88	44
85.0 (15 in 100)	128	64

**Indicates that the sample size is too small for a valid test.*

When nothing is worthy of special comments is found the results may be reported as *"The results of the field plot examination of this sample revealed nothing to indicate that varietal purity is unsatisfactory."*

Reporting of results

- The percentage of other species and cultivars of off-type plants must be indicated in the grow-out test findings.
- Results must be reported as such if the sample is discovered to be a cultivar different from the one specified by the sender.
- The report must specify that the sample contains a mixture of several cultivars if there are more than 15% plants from another cultivar.
- If no information deserving of special commentary is discovered, the report must mention that the sample's grow-out test findings showed nothing to suggest that the cultivar or species name provided by the sender is inaccurate.

Advantage:

- It is cheapest way to examine reasonable number of plants.
- It is possible to examine a large number of plots and for each plot it is possible to check large number of plants.

- The plants are examined during the whole period of growth. Some characters are more prominent at one time of the year than another, and the sample may therefore, be examined several times during the session.

Disadvantage:

- The result are not available until 4 to 12 months after the seed was receives for testing.
- The conventional grow-out test, which relies on morphological markers, is time- and space-consuming and frequently does not allow for the clear identification of genotypes. For a quick assessment of seed purity, molecular markers are crucial. Biochemical and molecular markers can be employed to support grow-out test results and mitigate their drawbacks.

References:

Agarwal, R. L. (2018). Seed Technology. India: *Oxford & IBH Publishing Company Pvt. Limited*.

Khare, D., Bhale, M. S. (2018). Seed Technology. India: *Scientific Publishers (India).*

Arus P. (2012). Genetic Purity of Commercial Seed Lots. *Developments in Plant Genetics and Breeding* Volume 1, Part A, Pages 415-423.

J. S. C. Smith and J. C. Register III (1998). Genetic purity and testing technologies for seed quality: a company perspective. *Seed Science Research*, 8, pp 285-294

*****

Seed Vigor Testing: Principles and Procedures

Abhinav Dayal
Department of Genetics and Plant Breeding, Naini Agricultural Institute
Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj

Seeds, as reproductive units, are expected to produce plants in the field. However, farmers and seed producers have long recognized that the labeled percent germination often overestimates the actual field emergence of seed lots. This observation is attributed to the objective of a standard germination test which states that germination is the emergence and development from the seed embryo of those essential structures which, for the kind of seed in question, are indicative of the ability to produce a normal plant under favorable conditions. (AOSA, 1991).

High-quality seeds play a crucial role in boosting crop production and overall productivity. Not only do they contribute to increased yields, but they also act as a preventive measure against the spread of diseases from one location to another. For farmers, investing in quality seeds translates to a substantial income boost, and it helps seed companies establish a stable presence in the market. The significance of quality seeds becomes evident when considering that they alone can bring about a remarkable 15-20% surge in productivity.

Ensuring the quality of seeds involves careful monitoring and testing across various parameters, such as genetic purity, physical purity, germination rate, seed vigor, moisture content, and overall seed health. This meticulous evaluation is essential before seeds are brought to the market and distributed to farmers. By focusing on these aspects, we not only enhance agricultural outcomes but also contribute to the livelihoods of farmers and the sustainability of seed companies. In essence, the journey from seed to harvest becomes a shared endeavor, highlighting the interconnectedness of farmers, seed producers, and the broader agricultural community.

Seed vigor testing, originating in 1876, shifted from early germination focus to evaluating factors influencing emergence and stand establishment. Seed vigour from the standpoint of seed testing, is the sum total of all seed attributes that favour stand establishment under varying field conditions. A vigour test is not a test for field response per se. The field response of a particular seed lot may more closely correlate with the vigour test or the ordinary laboratory test, depending upon the nature of field conditions under which planted. A vigour test, therefore, is an examination under specific environmental conditions so as to provide means of detecting differences that are not discernible in an ordinary laboratory germination test. The vigour test should be prognosticated in terms of field conditions which are critical with respect to the seed kind involved. The tests measuring those aspects of vigour which have little application to field problems, are of limited value.

Objectives of Seed Testing:

- **To determine the seed quality and evaluate seed viability:** Assess the viability of seeds to ensure their suitability for planting, focusing on the inherent quality that contributes to successful germination and robust plant growth.
- **To diagnose quality Issues:** Identify and analyze potential seed quality problems, delving into the root causes of any issues that may affect germination rates or overall crop performance.
- **To define labeling standards:** Establish precise labeling specifications that accurately convey essential information about seed quality, aiding farmers and stakeholders in making informed decisions during the selection process.
- **To establish quality benchmarks:** Set quality benchmarks that serve as a foundation for determining prices and facilitating consumer discrimination among different seed lots in the market. This ensures transparency and reliability in the seed industry.
- **To facilitate market differentiation:** Provide a reliable basis for distinguishing seed lots in the market, enabling consumers to make discerning choices based on established quality parameters. This promotes fair competition and ensures consumer confidence.
- **To guide drying and processing needs:** Determine the necessity for specific drying and processing procedures, offering valuable insights to seed processors and farmers to enhance the overall quality and performance of the seeds.

History of Seed Vigor

1. *Emergence of Seed Vigor Testing (1876)*

- The concept of seed vigor testing emerged in 1876 with Fredrich Nobbe introducing the term "triebkraft," emphasizing factors beyond germination in assessing seed quality.
- The focus was on the speed and uniformity of emergence, going beyond traditional germination metrics.

2. *International Seed Testing Congress (1950)*

- Concerns about non-standardized germination test results were raised during the 1950 ISTA Congress.
- Franck (1950) highlighted divergent approaches between European and American laboratories in germination testing.
- Franck proposed conducting germination testing under optimal conditions to ensure uniform results and introduced the term "vigor."

3. *Evolution of Seed Vigor Definitions*

- **1957 (Isely):** Seed vigor defined as "the sum total of all seed attributes favoring stand establishment under favorable conditions."
- **1960 (Delouche and Caldwell):** Refined definition emphasizing rapid and uniform stand establishment.
- **1965 (Woodstock):** Proposed vigor as "good health and natural robustness in seed," allowing rapid germination under various conditions.

- **1973 (Perry):** Identified seed vigor as a "physiological property" influenced by genotype and environment, governing rapid seedling production.

4. *ISTA Definition (1977)*
   In 1977, ISTA defined vigor as "the sum total of properties determining the potential activity and performance during germination and seedling emergence." Encompassed biochemical processes, growth rates, emergence ability under unfavorable conditions, and various influencing factors.
5. *AOSA Vigor Committee (1979)*
   AOSA's Vigor Committee (1979) provided an operational definition focusing on practical aspects: "seed properties determining the potential for rapid uniform emergence and development of normal seedlings under a wide range of field conditions."

Historical Progress in Seed Vigor Testing in the United States

1. *Initial Emphasis on Germination*
   Seed vigor testing in the U.S. began with a germination focus, considering vigor as more of a European concern.
2. *Forming of AOSA Vigor Test Committee (1961)*
   In 1961, the first AOSA Vigor Test Committee explored direct vs. indirect vigor tests and recognized the complexity of vigor testing.
3. *Breakthrough in Recognizing Seed Vigor (1974)*
   In 1974, acknowledgment of seed vigor's potential in estimating field performance led to the resolution to develop standardized seed vigor test procedures.
4. *Seed Vigor Definition and Progress Report (1980-1983)*
   By 1980, a seed vigor definition was approved, and procedures for seven useful vigor tests were published in the AOSA Vigor Testing Handbook in 1983.
5. *Accelerated Aging Test Advancements (1987)*
   In 1987, the accelerated aging test for soybeans moved from suggested to recommended status, showing advancements in vigor testing.
6. *Collaboration with ISTA and Education Efforts*
   Effective collaboration with ISTA and educational efforts led to increased adoption of vigor tests by laboratories, reflecting success in providing useful protocols and educating users.
7. *Reviews and Understanding Seed Vigor (Ongoing)*
   Ongoing reviews by experts continue to contribute to an enhanced understanding of seed vigor, emphasizing its importance as a key seed quality attribute influenced by various factors.

Seed Vigor Test:

Seed vigour, a single concept reflecting several characters determines the seed quality and uniform emergence potential of plants in field under variable range of environments. It was in 1876, when Nobbe first used the term 'Seed Vigour', thereafter, seed scientists are constantly digging in, to explore every possible scope this concept could provide.

Definition: "Seed vigour is a quantitative trait that is responsible for overall seed performance in terms of rate and uniformity of seed germination, seedling growth, emergence ability under unfavorable environments and post storage performance."

Principles of Vigor Test

Because of the complexity of factors influencing seed vigour and the wide variation in field conditions, no one vigour test is likely to serve best for all crops. The vigour tests, therefore, should be crop specific and a combination of methods) may be preferred in specific cases as compared to a single test method. It should be based on the following criteria:

1. Reproducibility of results, which could be objectively as sessed, clear and meaningfully interpreted.

2. The correlation with the field stand, that is, seedling emergence.

3. It should be based on sound principles.

4. It should be simple and cheap to perform on a large numbe of samples in rapid succession. The ultimate proof of any vigour test is its reliability in predicing field stands under a variety of field conditions.

Importance of Seed Vigor:

i. **Viability and Growth**: It measures not only the percentage of viable seeds in a sample but also their ability to produce normal seedlings under less than optimum or adverse growing conditions.
ii. **Field Conditions**: Seeds may be classified as viable in a germination test which provides optimum temperature, moisture, and light conditions to the growing seedlings; however, they may not be capable of continuing growth and completing their life cycle under a wide range of field conditions. Also, Seed vigour evaluation helps in monitoring seed physiological potential during various stages of seed production
iii. **Storage Potential**: Seed vigour testing is used as an indicator of the storage potential of a seed lot and in ranking various seed lots with different qualities.
iv. **Seed Longevity**: Vigour determines seed longevity as well as speed and uniformity of seed germination and emergence.
v. **Overall Performance**: Seed vigour is responsible for overall seed performance in terms of rate and uniformity of seed germination, seedling growth, emergence ability under unfavorable environments, and post-storage performance.
vi. **High Quality Seeds:** Seed vigour evaluation assists in strategic decisions about the selection of high-quality seed lots to fulfill market demand.

EFFECTS OF SEED VIGOUR

Vigour of seeds expresses itself in, broadly, four ways:

(i) Survival intact in the non-active state: a vigorous seed is one that remains vigorous.

(ii) Survival upon sowing in the field: a vigorous seed resists or out-strips its attackers.

(iii) Ability to establish a plant: a vigorous seed has plenty of all the right reserves, and it uses them during the heterotrophic and transition phases of growth.

(iv) Ability to grow well: a vigorous seed results in a seedling that grows vigorously during the autotrophic phase of growth.

Factors Influencing Seed Vigor

The development of a seed undergoes various ontogenetic stages, from fertilization to seed dry down and dormancy. Physiological maturity, the point at which a seed achieves its maximum dry weight, offers the greatest potential for maximum germination and vigor. However, seeds are typically not harvested until they reach harvest maturity, which is low enough for safe storage but high enough to minimize mechanical injury. Between physiological maturity and harvest maturity, seeds are vulnerable to environmental conditions that can adversely affect seed quality.

Several factors influence seed vigor, including genetic constitution, the environment during seed development, and the seed storage environment.

1. **Genetic Constitution:**

- Plant breeders, in their efforts to increase yields, inadvertently select for increased seed vigor. Improved characteristics such as mechanical integrity, disease resistance, protein content, and seed size contribute to better field emergence and enhanced yields.
- Hybrid vigor, a measurable superiority of hybrid progeny over inbred parents, enhances growth potential, especially under stress conditions.
- Traits like hard-seededness, susceptibility to seed damage, and seed chemical composition under genetic control also impact seed quality.

2. **Environment During Seed Development:**

- Seed production concentration in specific areas, influenced by environmental conditions, is crucial for high-quality seed production.
- Soil moisture and fertility, seed maturity, and the post-maturation pre-harvest environment are factors affecting seed quality.
- Moisture stress during seed development can result in light, shriveled seeds, leading to poor-vigor seed.
- Soil fertility, though generally not limiting for seed viability, can influence seed vigor.
- The environment during seed maturation indirectly affects potential seed vigor, with larger seeds generally exhibiting better germination and vigor.

3. **Post-maturation and Pre-harvest Environment:**

- Deterioration of seeds during this period is a significant issue in regions with high humidity, frequent rainfall, and warm temperatures.
- Exposure to rainy conditions can lead to a rapid loss in seed viability.
- Delayed harvest due to inclement weather can result in lower seed quality and increased mechanical damage during harvest.
- Pre-harvest loss of seed quality is exacerbated by fungal invasion, particularly in warm and humid conditions.

4. **Mechanical:** gross breakages or the creation of necroses which may spread through physiological mechanisms.
5. **Microbial:** the burden of fungi and/or bacteria which accumulate on and in the seed during its maturation may endanger its performance under appropriate storage or field conditions by heating in store, by direct attack, including the invasion and enlargement of necroses, or by competition for oxygen. (Heydecker and Chetram, 1971).

Challenges in Seed Vigor Testing

Identifying Quantifiable Parameters for Deterioration

- The challenge in seed vigor testing lies in identifying common, quantifiable parameters associated with seed deterioration.
- Delouche and Baskin's model (1973) outlines major parameters for measuring seed vigor.
- Vigor tests, more sensitive than standard germination tests, focus on events preceding germination loss.

Categories of Vigor Tests

- Direct and indirect tests
- Physiological and biochemical tests
- Stress and quick tests

Physiological and Biochemical Implications

- **Direct Tests:** Simulate field conditions (e.g., cold test).
- **Indirect Tests:** Measure specific physiological components (e.g., conductivity).
- **Physiological Tests:** Focus on germination-related parameters.
- **Biochemical Tests:** Evaluate chemical reactions related to germination.

Characteristics of Effective Vigor Tests

- **Affordability:** Crucial due to limited testing budgets.
- **Rapidity:** Benefits seed producers in competitive markets.
- **Simplicity:** Ensures broader applicability.
- **Objectivity and Reproducibility:** Enhance standardization for consistent interpretation.
- **Correlation with Field Performance:** The ultimate value, validating practical utility.

Types of Seed Vigor Tests

The standard germination test, conducted under optimal conditions, correlates well with field emergence when planting conditions are near optimum. However, under suboptimal conditions, standard germination results tend to overestimate field emergence. To address this limitation, additional vigor tests have been developed, some of which are widely accepted in seed testing organizations:

1. **Cold Test:**
- **Purpose:** Evaluates seed vigor, especially in corn and soybeans.
- **Method:** Seeds are subjected to cold stress in soil or paper towels, followed by favorable growth conditions for germination.
- **Challenges:** Variability in field soil conditions may affect results.
2. **Accelerated Aging Test:**
- **Purpose:** Initially designed for seed storability assessment.
- **Method:** Seeds undergo short-term exposure to high temperature and humidity, followed by optimal germination conditions.
- **Advantages:** Rapid, inexpensive, applicable to all species, and has been standardized.
3. **Conductivity Test:**
- **Purpose:** Measures membrane integrity, detecting cytoplasmic solutes released during imbibition.
- **Method:** Rapid, precise, and inexpensive; measures electrical conductivity of leachates.
- **Considerations:** Seed moisture and size may impact results, and antibiotic treatment may influence measurements.
4. **Cool Germination Test:**
- **Purpose:** Assesses seed vigor under standard laboratory conditions at low temperatures.
- **Advantages:**Similar to standard germination test, applicable to cotton.
- **Limitation:** Currently limited to use in cotton.
5. **Seedling Growth Rate Test:**
- **Purpose:** Evaluates seedling growth rate based on dry weight accumulation.
- **Method:** Growing segments of embryos from normal seedlings are weighed after drying.
- **Considerations:** Requires standardization for specific cultivars; influenced by moisture and light intensity.
6. **Seedling Vigor Classification Test:**
- **Purpose:** Expands routine germination test, classifying "normal" seedlings into "strong" and "weak" categories.
- **Advantages:** No additional equipment required, familiar concepts to seed analysts.
- **Challenge:** Subject to interpretation difficulties; may require referee programs for standardization.
7. **Tetrazolium (TZ) Test:**
- **Purpose:** Analyzes seed quality based on formazan formation from dehydrogenase enzyme activity.
- **Method:** Subjective evaluation by a trained analyst; categorizes seeds into vigor levels.

- **Challenges:** Subject to standardization difficulties; may not detect seed treatment phytotoxicity or reveal seed dormancy.

8. **Speed of Germination:**

- **Purpose:** Assesses the rate of germination, providing an index of seed vigor.
- **Method:** Variations in the number of days required to reach a specified percentage of germination.
- **Advantage:** Little additional work compared to the standard germination test.

9. **Brick Grit Test:**

- **Purpose:** Originally developed for detecting Fusarium infection; also used for assessing seed vigor.
- **Method:** Seeds planted on damp brick grit; inability to penetrate indicates seed weakness.
- **Challenges:** High cost, space requirements, variability in results, and difficulties in obtaining, washing, and drying brick grit.

10. **Osmotic Stress Test:**

- **Purpose:** Simulates drought stress conditions in a laboratory setting.
- **Method:** Seeds germinated in solutions with specific osmotic potentials.
- **Advantage:** No special equipment or training required; simulates drought stress.

11. **Respiration Test:**

- **Purpose:** Measures the rate of respiration during germination.
- **Method:** Rapid and quantitative, using a respirometer.
- **Considerations:** Requires trained personnel; mechanical injury may impact results.

These tests aim to provide a more accurate assessment of seed vigor under a range of conditions, supplementing the standard germination test, especially in predicting field emergence under suboptimal conditions. Each test has its advantages and limitations, and the choice depends on factors such as seed type, resources, and testing objectives.

Factors Affecting Reproducibility of Seed Vigor Tests

1. Subjectivity in Tests:

- Many vigor tests involve subjective interpretations, making standardization challenging. For instance, the seedling vigor classification and tetrazolium vigor tests require interpretations based on characteristics that are difficult to precisely describe.

2. Environmental Conditions:

- Variations in temperature, moisture, and other environmental conditions are critical, particularly in tests measuring the rate of growth or a biochemical process. Conditions suitable for standard laboratory germination tests may not be suitable for vigor tests.

3. Cold Test Standardization:

- The cold test, in its present state, is challenging to standardize because it exposes seeds to soil microorganisms. Standardization of the cold test procedure requires standardization of the substrate (soil) microflora, a task that is difficult. Suggestions like using sterile media inoculated with specific microorganisms face challenges.

Dormancy's Impact on Vigor Test Results

Factors such as dormancy can affect vigor test results, especially in species with impermeable or slowly permeable seeds. Dormancy can lead to biased results in stress tests and conductivity tests. To address dormancy-related issues, standard warm germination tests or tetrazolium tests are recommended.

Challenges in Standardization

Despite these challenges, a study by Byrum and Copeland in 1995 indicated that the cold test for corn in the U.S. corn belt is as repeatable as the standard germination test. This study demonstrated the potential for the eventual standardization of all vigor tests.

Efforts toward Standardization

While vigor testing for all species and regions has not achieved the same level of standardization as the standard germination test, efforts are being made by seed-testing organizations and the seed trade to improve reproducibility.

Relationship between Seed Vigor and Final Yield

High-vigor seeds may provide better field emergence, but the relationship between high-vigor seeds and final yield is not as well-established, requiring further research. Overall, while standardization efforts are ongoing, the promise of vigor testing in the future is optimistic.

IMPORTANCE OF SEED TESTING

The AOSA Seed Vigor Testing Handbook (1983) outlines various uses of seed vigor tests. Farmers can benefit from high-vigor seeds, especially in adverse soil conditions or when adverse environmental conditions are expected after planting. Seed companies often conduct a series of vigor tests, combining information into a seed vigor index.

(a) The importance of seed testing focuses on the procedures for assured planting values.

(b) It's an aid for seed industry 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.

(c) Quality control of seed depends on the different seed testing protocols to determine the genuineness of the cultivar.

(d) Testing of seed to evaluate the planting value and the authenticity of the certified lot.

(e) Assessment of the seed quality attributes of the seed lots which have to be offered for sale.

(f) 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 ISTA.

(g) Economic yield of a crop depends on the quality of seeds which can be evaluated by seed testing (ISTA, 1996).

Conclusion

The evolution of seed vigor testing has been a journey from early germination-centric approaches to a nuanced evaluation of factors influencing seed emergence. Despite challenges in quantifying deterioration parameters, seed vigor tests, such as the cold test and tetrazolium test, offer valuable insights, especially in adverse conditions. Collaborative efforts toward standardization are underway, holding the promise of improved reproducibility.

Seed vigor testing is playing a crucial role in agricultural practices, aiding farmers in adverse conditions and ensuring seed quality for companies. Ongoing research exploring the link between seed vigor and final yield highlights the potential for future advancements in seed testing practices and agricultural strategies.

The future of seed vigor testing is poised for exciting developments, driven by advancements in technology, a deeper understanding of seed biology, and the ongoing need for sustainable agricultural practices. These trends will contribute to the resilience and adaptability of crops in the face of evolving environmental challenges, ultimately supporting global food security and agricultural sustainability.

*****

Seed Germination: Principles and Procedures

Smt. Ekta kumari
Sr. Seed Analysts, National Seed Research and Training Centre, Varanasi

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 **s**tatutory 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 $\pm$ 2^0C. 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 forgermination 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 40^0C 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	*Echinocloafrumentacea*	TP	20-30	4	10	Prechill, KNO_3, GA_3
Finger Millet	*Elusinecoracane*	TP; BP	20-30	4	8	0.2% KNO_3 (2-3 hrs)
Kodo Millet	*Paspalumscorbiculatum*	TP	20-30	7	20	KNO_3
Pearl Millet (Bajra)	*Pennisetumtyphoides*	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	*Viciasatva*	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	*Lininumusitat*	TP; BP	20-30;20	3	7	Prechill

	ssimum					
Mustard	*Brassica juncea*	TP	20-30;20	5	7	Prechill, KNO_3
Mustard (Black)	*Brassica nigra*	TP	20-30;20	5	10	Prechill, KNO_3
Niger (Ramtil)	*Guizotaabyssinica*	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)	*Cenchrussetigerus*	TP	20-35	3	14	Preheat (40^0C)
Buffel grass	*Cenchruscilliaris*	TP;S	20-35	7	28	Preheat ; Prechill, KNO_3
Burmuda grass (Doob)	*Cynodondactylon*	TP	20-35	7	21	Prechill, KNO_3; Light
Dharaf grass	*Andropoganmontanus*	TP	20-35	7	28	Prechill at 5^0C for two weeks
Dinanath grass	*Pannisetumpedicellatum*	TP	35;20-35	7	28	H_2SO_4fro 5 min
Guinea grass	*Panicum maximum*	TP	15-35;20-30	10	28	Prechill, KNO_3
Indian clover (Senji)	*Melilotusindica*	TP;BP	20	4	7	Prechill
Lucerne	*Medicago sativa*	TP;BP	20	4	10	Prechill
Marvel grass	*Dichanthiumanulatum*	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	*Loliumparenne*	TP	20-30	5	14	Prechill ; KNO_3
Sataria grass (Nandi grass)	*Setaria anceps*	TP	20-35	7	21	KNO_3
Stylo	*Stylosanthussp p*	TP	20-35	4	10	H_2SO_4
Sudan grass	*Sorghum sudanense*	TP;BP	20-30	4	10	Prechill
Teosinte	*Euchlaenamexicana*	BP;S	20-30;25	-	7	GA_3 1000 ppm – 24 hrs

GREEN MANURE AND MISCELLANEOUS CROPS						
Dhainch	*Sesbaniasp*	TP;BP	20-30	5	7	Rub seed coat on sand paper
Indigo	*Indigoferahirsuta*	BP	20-30	-	14	Continue test for a further 5 days if hard seeds have begun to imbibe
Chicory	*Cichoriumintybus*	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	*Benincasehispida*	S	30-35	5	14	Light
Pointed gourd	*Trichosanthusdioica*	S	30-35	-	14	Dark, GA_3 500 ppm 24 hrs, Remove seed coat
Snakegourd	*Trichosanthusanguina*	S	30-35	-	14	Dark, GA_3 500 ppm 24 hrs, Remove seed coat
FRUIT VEGETABLES						
Chilli	*Capccumspp*	TP ; BP	20-30	7	14	KNO_3
Tomato	*Lycopercicumesculentum*	TP ; BP	20-30	5	14	KNO_3
BULB AND TUBER CROPS						
Leek	*Allium porrum*	TP ; BP	20-15	6	14	Prechill
Lesser yam	*Dioscoraspp*	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	*Apiumgraveolens*	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	*Viciafaba*	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; swol-len 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 *Phomabetae)* 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 Gramineae 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** ***Tetragoniaexpansa,*** schizocarps of*umbelliferae*, and multiple florets of *Chloris gayana, Arrhanatherumelatius, Dactylisglomerata,* 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.

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

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.

*****

Biochemical Tests for Varietal Purity Testing in Seeds

Abhinav Dayal
Department of Genetics and Plant Breeding, Naini Agricultural Institute
Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj

Seed, as the fundamental input in agriculture, plays a pivotal role in driving production, productivity, and a nation's economy. Ensuring timely access to quality seeds at reasonable prices for the farming community is crucial to meet the growing needs of the population. In the context of cross-pollinated crops and the adoption of hybrid varieties, it serves as an alternative strategy to maximize production and productivity.

Quality seeds possess the inherent capability to optimize resource utilization, including fertilizers and irrigation, contributing to food security. The significance of quality seeds extends beyond achieving higher yields; it directly correlates with increased monetary returns. Quality seeds alone can enhance production, and when strategically combined with other inputs, they contribute to elevated yield levels.
Several factors influence the production of quality seeds, encompassing genetic purity, physiological quality, and the presence of contaminants like weed seeds and seed-borne diseases. Genetic purity, defined as the percentage of contamination by seeds or genetic materials of other varieties or species, is critical for commercial agricultural products propagated by seed. The genetic purity journey commences with the purity of the seed planted.

Throughout the seed production process, various factors may lead to the deterioration of genetic purity. These include developmental variations, mechanical mixtures, natural crossing, genetic drift, the influence of diseases, and mutations. The introduction and commercial cultivation of genetically modified (GM) crops pose challenges, particularly in terms of contamination affecting non-GM and organic crops. Addressing genetic deterioration necessitates a multi-faceted approach, involving molecular techniques, traditional genetic purity testing, chemical methods, control plot strategies, quarantine measures, and cultural methods.

While morphological methods, such as assessing plant height, seed size, and color, are commonly employed, they fall short in providing precise information about grain quality, especially regarding specific genetic attributes like resistance to pests or herbicides. Biochemical assays and isozymes have been traditionally used for distinguishing varieties within species like maize. However, the emergence of newer DNA-based technologies, including molecular markers, holds great promise for enhancing the assessment of seed purity. These technologies allow for clear differentiation and expedited identification of varieties.

Seed Quality

At its core, a seed encompasses not only the rudimentary plant parts of stems, modified stems, and true seeds but also represents the bedrock for the commercial amplification of crops. A seed, strictly defined, manifests as a fertilized ovule that houses an embryonic plant, stored nourishment, and a seed coat endowed with germination potential. Within the intricate framework of seeds lies genetic material, whether inherent or engineered through breeding, which forms the essential conduit for passing on

desirable traits from one generation to the next. The broad spectrum of seed quality encapsulates genetic purity, physiological purity, physical purity, and seed health.

Genetic Purity

Genetic purity, quantified as the percentage of contamination by seeds or genetic materials of other varieties or species, assumes a pivotal role in both plant breeding and seed production. Its significance lies in ensuring trueness to type and the exclusion of undesired genetic variations. Traits emblematic of genetic purity span plant type, growth cycle duration, seed color, seedling vigor, resistance to pests and diseases, and specific grain or seed qualities. In the realm of cross-pollinated crops, genetic purity emerges as a linchpin for the success of hybrid seed production.

Importance of Quality Seed

Seeds, undeniably, serve as the bedrock of agriculture, their quality wielding a direct and decisive impact on crop production and plant breeding. High-quality seeds not only facilitate the judicious use of agricultural inputs, fostering increased economic returns but also become indispensable for meeting the burgeoning needs of a growing population and elevating overall production and productivity. The optimization of fertilizers and irrigation, underscored by genetic purity, not only ensures higher yields but also augments monetary returns.

Genetic Purity Characteristics

A meticulous evaluation of genetic purity encompasses an array of dimensions, including physical attributes, chemical analysis, and crop factors. Genetically pure seeds are distinguished by specific traits such as length, width, shape, size, color, and aroma. Chemical analyses, involving tests for amylase content, alkali digestion color, and gel consistency, serve as discriminators between varieties. Crop factors, encompassing plant height, time to maturity, plant color, and growth habit, further contribute to the assessment of genetic purity.

Genetic Purity Deterioration

Genetic purity deterioration signifies the irreversible reduction, either genetic or agronomic, of a given crop. This deterioration could commence at any stage, spanning storage to the plant's developmental stages from sowing to threshing. The quality of the seed is intrinsically tied to the physical conditions experienced by the mother plant across different environmental conditions during its growth stages, harvest, processing, storage, and planting. Bradford (2006) asserts that the genetic purity of the seed planted must either match or surpass the final product's required purity standard, given that purity generally wanes with each subsequent generation of propagation.

Causes of Genetic Purity Deterioration

The pursuit of seed production with robust genetic purity encounters several challenges. Developmental variation unfurls when a seed variety is cultivated beyond its native environmental conditions for consecutive generations. Mechanical mixtures may arise at any development stage, spurred by factors such as the use of the same planter for distinct varieties or the co-cultivation of different varieties. Natural crossing is commonplace in sexually propagated crops, while genetic drift gains traction in expansive seed crop areas where only a minute quantity of seed is conserved for subsequent sowings.

The influence of diseases, mutations, the techniques employed by plant breeders, and the advent of genetically modified (GM) crops further exacerbate genetic purity deterioration. GM crops, in particular, introduce unique challenges due to potential contamination of non-GM and organic crops. The intricate web of factors contributing to genetic purity deterioration underscores the need for vigilant monitoring and mitigation strategies.

Maintaining Genetic Purity

Countering the ominous specter of genetic purity deterioration demands a multifaceted approach:

1. **Crop Rotation:** Land chosen for seed production must be devoid of the same crop species for at least one or two years, minimizing the risk of genetic contamination.
2. **Isolation Distance:** Establishing a spatial gap between seed crops and other varieties serves as a bulwark against cross-pollination and mechanical mixtures. The isolation distance is contingent on the crop's pollination behavior.
3. **Field Inspection:** Continuous scrutiny during the growing season proves instrumental in identifying and excising off-types and diseased plants, curbing genetic contamination.

Genetic Purity Control

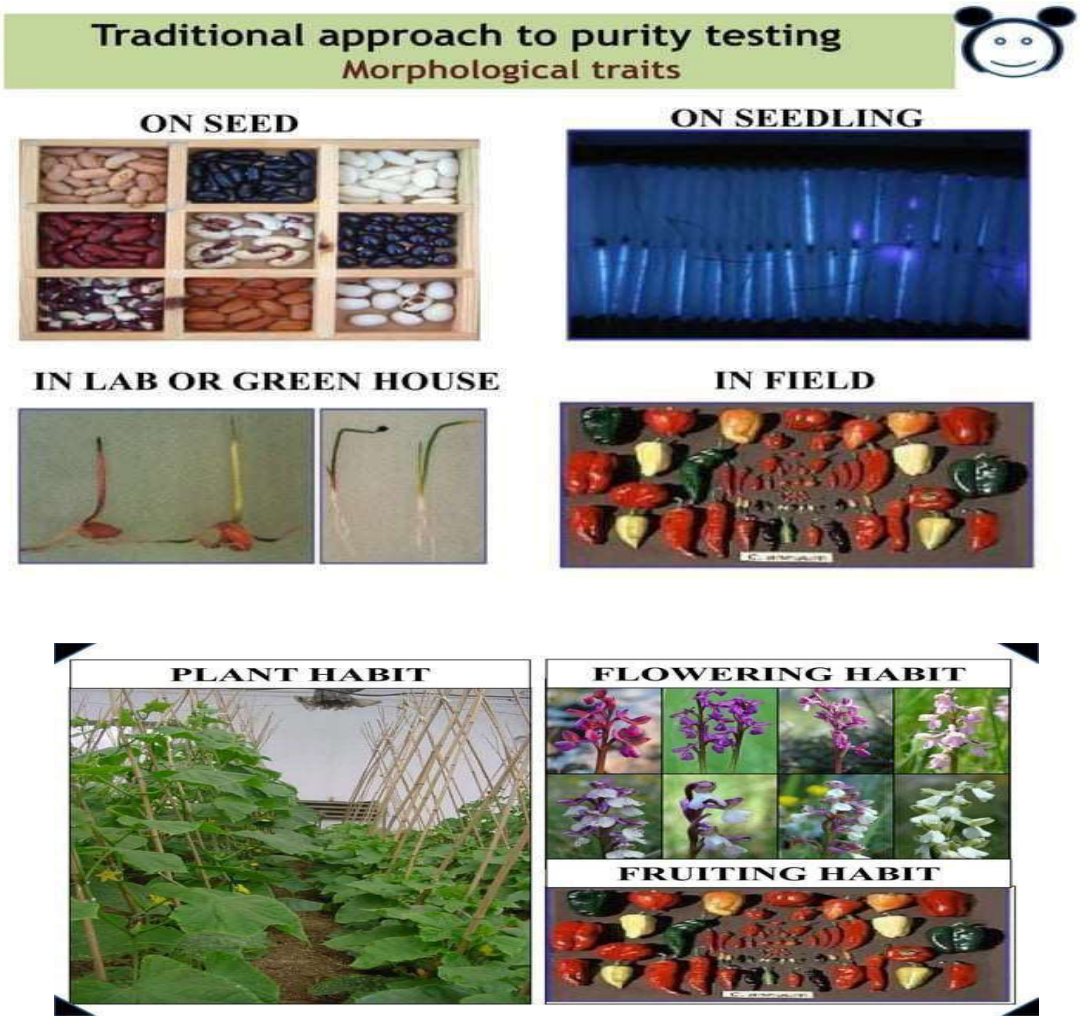
1. **Seed Certification:** The rigorous process of evaluation, encompassing field inspection and laboratory analysis, culminates in the certification of high-quality seeds and propagating materials. Certification standards undergo tailoring to suit diverse crops.
2. **Seed Legislation:** Legal frameworks regulating seed quality and marketing play a pivotal role in safeguarding both consumers and producers. Legislation assumes heightened significance, especially in the context of genetically modified organisms (GMOs).

Genetic Purity Testing

The labyrinthine task of assessing genetic purity spans diverse methods:

1. MorphologicalMethods:

- Genetic purity evaluation can be conducted at various stages including on seeds, seedlings, in laboratories, green houses, and in the field.
- Morphological methods, such as evaluating plant habit, head color, and shoulder shape, offer insights in to varietal characteristics.
- Traits like plant height, seed size, and colour lack specificity for assessing genetic attributes like pest or herbicide resistance.
- Environmental factors canal so alter the expression of these multigenic traits. To overcome these limitations, rapid and reliable chemical tests are suggested to expedite the process, particularly for chili genotypes.



2. ChemicalMethods

Chemical methods for testing genetic purity include various tests like Phenol, Ferrous sulphate, Fluorescence test, and others. These tests leverage chemical reactions or compounds within seeds to differentiate varieties. For instance, phenol tests have been used for cultivar identification in wheat, barley, oat, ryegrass, and bluegrass. Chemical methods offer advantages such as quick results, affordability, simplicity, and the ability to detect admixture percentages. Some chemical methods listed below

1. Phenol test
2. Ferrous sulphate test
3. Fluorescence test
4. 2,4-D soak test
5. Peroxidase test
6. Potassium hydroxide (KOH) test
7. KOH bleach test
8. Hydrogen peroxide (H_2O_2) test
9. Sodium hydroxide (NaOH) Test
10. Gossypal test.
11. Amylose content in endosperm
12. Oil content
13. Erucic acid content in seeds

14. Lugols test

Biochemical and Imaging Technique for Analysis (BITA)

Isozyme analysis and electrophoresis of proteins can be employed for genetic purity determination, although isozyme analysis has limitations due to factors affecting expression. Electrophoresis of proteins, particularly blotting from iso-electrically focused gels, shows promise in differentiating parental lines. These biochemical and imaging techniques offer valuable insights into varietal differences, contributing to the maintenance of genetic purity.

Conclusion

The imperatives of sustaining agriculture hinge on the preservation of seed genetic purity. As seeds constitute the foundational elements of crop production, judicious efforts must be channeled toward ensuring their genetic integrity. Simple, cost-effective, and applicable methods, whether standalone or in combination, emerge as the bedrock for accurate genetic purity control. The pervasive specter of genetic deterioration necessitates ongoing vigilance, with chemical methods, molecular techniques, and biochemical and imaging analyses serving as indispensable tools in the pursuit of seed quality evaluation.

In conclusion, the dynamic interplay between genetics, environment, and agricultural practices underscores the need for a holistic and adaptive approach to seed quality management. The evolving landscape of genetic technologies and their implications on seed production necessitates continuous research and refinement of genetic purity testing methods. This chapter lays bare the multifaceted challenges and potential solutions, casting a spotlight on the intricate dance between genetic purity and sustainable agriculture.

*****

Detection and Identification of Insect Infestation in Seed Lots

Smt. Ekta Kumari
Sr. Seed Analyst,
National Seed Research & Training Centre, Varanasi

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,	*Sitophilusoryzae* (Curculionidae, coleoptrea)	Rice, wheat, sorghum, barley, maize
Lesser grain borer	*Rhizoperthadominica* (Bostrychidae, coleoptera)	Rice, wheat and maize
Pluses beetle	*Callosbruchuschinensis, C. maculates*, (Bruchidae, Coleoptera)	Pulses, bean and gram.
Cigarette beetle	*Lasiodermaserricorne*, (Anobiidae, Coleoptera)	Tobacco, wheat, peanut , cocoa, bean, cotton seed
Drug store beetle	*Stegobiumpaniceum*, (Anobiidae, Coleoptera)	Turmeric , coriander, ginger, dry vegetables and animal matters
Groundnut bruchid	*Caryedon serratus* (Bruchidae, Coleoptera)	Ground nut and other legumes
Angoumois grain moth	*Sitotrogacerealella*, (Gelechiidae, Lepidoptera)	Paddy, maize and wheat.
Potato tuber moth	*Phthorimaeaoperculella* (Gelechiidae, lepidoptera)	Potato, tomato, tobacco, eggplant and *Betavulgaris*
Sweet Potato weevil	*Cylasformicarius*, (Apionidae, Coleoptera)	Sweet potato few other species of *Ipomoea*

EXTERNAL FEEDERS

Common name	**Systematic position**	**Hosts**
Khapra beetle	*Trogodermagranarium*, (Dermestidae, Coleoptera)	Cereals and groundnut pulses species and pulses cakes
Red flour beetle	*Triboliumcastaneum, T. confusum*, (Tenebrionidae, Coleoptera)	Maize wheat, etc.
Saw toothed grain beetle	*Oryzaephilussurinamensis*, (Silvanidae, Coleoptera)	Dry fruits, rice , wheat, maize cereals and oilseeds
Rice moth	*Corcyra cephalonica*, (Galleridae,	Cereals, oilseeds nuts, dry

	Lepidoptera**)**	fruits, rice and pulse
Tropical Warehouse Moth.	*Ephestiacautella* (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, Sitotrogacerealella*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.
- **Godownsitself :**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	*Callosobruchuschinensis*	Stored pulses	'Erection', a mixture of hydrocarbons, dicarboxylic acid	M/MT
Lesser grain borer	*Rhyzoperthadominica*	Stored cereal	Dominicalure 1: (S)-(+)-1-methyl butyl (E)-2, butyl (E)-2, 4-dimethyl -2-pentenoate	M/MT
Grain moth	*Sitotrogacerealella*	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,

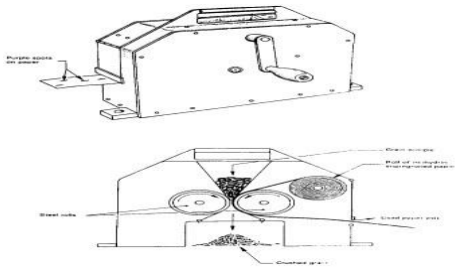
The mucilaginous secretions of weevils (*Sitophilus* sp.) are stained with a suitable chemical compound. Weevils deposit their eggs inside the seed and plug the holes or egg cavities with saliva. Using suitable coloring agents, the egg plugs in grains can be stained and identified. The extent of infestation 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)

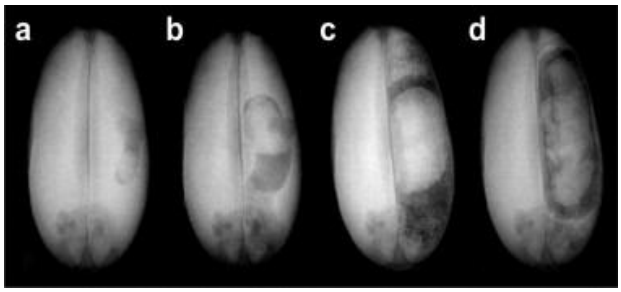
The ninhydrin method : The chemical indicator, ninhydrin (triketohydrindene hydrate) is used for detection of live insects inside the seed. When insect body fluid comes in contact with the ninhydrin-impregnated filter paper, it produces a "purple" colour.

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

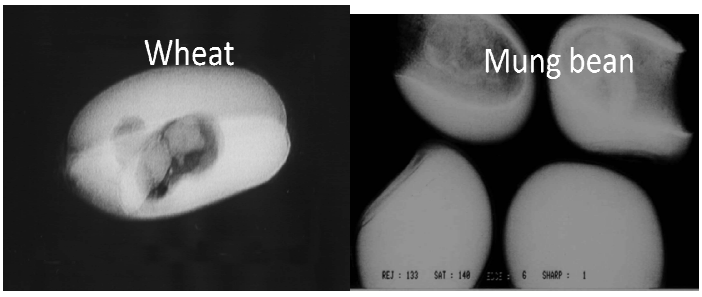


Apparatus for ninhydrin detection of hidden insect infestation "Ashman-Simon infestation detector"

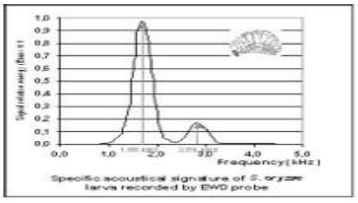
5. X-ray method: This is an accurate and rapid method of detecting internal insect infestation in a sample of seed and was described by (Milner et al., 1950). An X-ray machine operates at about 50 KW. Exposure period varies from seed to seed to get a good radiograph which reveals the presence of any 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.

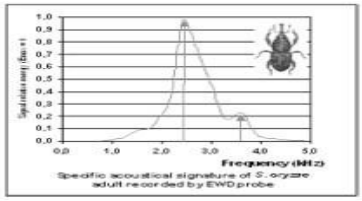


X-ray images of wheat seed infested by different stages of granary weevil (*Sitophilus granaries)*: small larva (a), medium larva (b), large larva (c) and pupae (d).



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.

*****

Management of Insect Infestation in Seed Lots

Ekta Kumari
Sr. Seed Analyst,
National Seed Research & Training Centre, Varanasi

Management of insect infestation in seed lots can be divided into two broad areas based on the type of intervention followed. They are

1. Preventive measures and
2. Curative measures

1. **PREVENTIVE MEASURES:**

The following preventive measures are recommended:

a. Sanitation and handling of seed lots

Removing dirt, debris, mud balls, foreign particles, insects and infested grains from healthy grains is a common practice followed by the objective of minimizing storage losses. This has been shown to reduce insect infestation (Pillayar, 1979). Seed lot bags should be stacked on wooden dunnage 0.5 meters away from the wall, above the ground and stock to stock. Bags should be stacked in *rows* having a space of nearly 2 to 3 meter in-between and height of a row should not be more than 15 bags 1eavng about 1/5 space of total storage from the roof.

b. **Drying**

The moisture content of the seed above the minimum level required for insect development (12-13 percent) results in storage losses. Sun drying and the use of mechanical dryers can be opted to bring moisture down. Improper drying of paddy grains during post harvest operations not only enhances the insect infestation, but also enhances breakage during milling. Staggered sun drying with short exposure to sun spread over large number of days (9-11 am for 8 days) reduces insect infestation.

c. Use of improved storage structures

Gunny bags or jute bags with close weave can reduce insect infestation. Impregnation of gunny bags with insecticides can prevent entry of insects (Prakash et al., 1981). Polythene lined gunny bags were suggested by Muthu and Pingale, (1955). Polyester- polythene 400 gauge lined canvas was found to be resistant to all types if insect attack. Improved storage structures, namely aluminum bin, Pusa bin, Pusa cubicle PAU bin, IGSI domestic bin, TNAU insect removal bins have been found very effective for bulk storage and reducing insect damage.

d. Disinfestation of stores/receptacles

Treatment of bulk and bag storage structures with insecticides is an important practice to avoid latent infestation in reused bags and bulk storage structure. The insecticides commonly recommended are malathion and dichlorvos.

e. Legal method

In India, the Destructive insects and Pests Act 1914 and its latest amendment, the Plant Quarantine Order 2003, govern the regulation or restriction of movement of insects through commodities in the country and among different areas within the country.

2. CURATIVE MEASURES

a. Physical control measures

The infestation of stored grains by insect pests largely depends on the three factors, temperature, moisture content of grain, availability of oxygen. All these factors are required for normal development and multiplication of insects. Hence, they have to be properly manipulated through design and construction of storage structures/godowns and storage practices so as to create physical conditions unfavorable for attack by insects.

i. Use of low and high Temperatures

The insects can be controlled either by increasing or decreasing storage temperature. The optimal temperature for most of the storage insects is between 25 and 33^{o} C. Temperatures between 13 and 25^{o} C will slow development. High temperatures of 35^{o} C and above will stop development. High temperature disinfestations using heated air grain driers, fluidized beds, spouted beds, pneumatic conveyors, a counter flow heat exchanger, high frequency waves, microwaves, infra red waves and solar radiations have been satisfactorily used for in disinfecting grains.

ii. Mixing of inert dust

Many diatomaceous earth (DE), dusts is now commercially available and used for managing stored-product insects and mites, or to improve fumigation efficiency. Activated clay (kaolin) has been used in protecting seeds from the attack of storage insects. This method is very effective against most of the storage pests and nontoxic to higher animals. The other inert dusts used in stored-product protection can be categorized into 4 groups.

1st group	Clays, sand, paddy husk ash, volcanic ash and wood ash(shown to posse insecticidal properties)
2nd group	Dolomite, magnesite, copper oxychloride, rock phosphate and ground sulfu lime (calcium hydroxide), limestone (calcium carbonate), and common sa (sodium chloride).
3rd group	Dusts that contain synthetic silica (silicon dioxide).
4th group	Dusts that contain natural silica, such as diatomaceous earth (DE), which a made up fossilized skeletons of diatoms

iii. Irradiation: Low dose irradiation completely kills or sterilizes the common grain pests, and even the eggs deposited inside the grains. Moreover, only a single radiation exposure of grains is sufficient for disinfestations. This, therefore, is ideally suited for large-scale operations, thereby offering substantial economic benefits.

iv. Use of controlled atmosphere

In seed storage, insects can be controlled by decreasing O_2 or increasing CO_2 or N_2 concentration in the atmosphere, thereby interfering with the normal respiration of insects. This is achieved by modified atmospheric storage, controlled atmospheric storage or airtight storage. In case of modified atmosphere, the storage atmosphere is modified by introducing CO_2 or N_2 replacing O_2. Controlled atmosphere is precisely maintaining the composition of selected gases such as CO_2, O_2 and N_2 at specified concentration under

normal pressures or under partial vacuum. Airtight or hermetic storage of grains/seeds lead to decrease in available O_2 and increase in CO_2 due to respiration and metabolism of the seeds.

b. Mechanical control measures

The mechanical control methods are quite practicable. Several mechanical devices have been designed and developed both for monitoring and mass trapping stored product insects. They are insect probe trap, pitfall trap, two-in-one trap for pulse beetle, indicator device, automatic insect removal bin, UV – Light trap for warehouse and stored grain insect pest management kit, sticky traps, bait traps and pheromone traps. All these devices can be used for both monitoring and mass trapping of stored grain insects.

c. Use of plant products

The powders of the leaves of Neem, and Nochi, Vitex *negundo* when mixed with seeds gives protection from insects.The fresh leaves of Begunia mixed with paddy at the rate of 2% w/ protected the grains from insect attack for 9 months. Garlic extract is yet another plant product which is nontoxic and was found to be seed protectant. Neem leaf powder, turmeric powder, Sweet Flag Rhizome powder all at 10g /kg have been found to be effective against seed storage pests.

II. Chemical control measures

a. Prophylactic treatment

1. If the produce is meant for seed purpose, mix 1 kg of activated kaolin or malathion 5% D for every/100 kg of seed and store/pack in gunny or polythene lined bags.
2. Apply one of the following pesticides at the specified dosage over the bags. Malathion 50 EC : 10 ml per litre of water and 3 litres of spray solution per 100 sq.m. (or) DDVP 76% SC : 7 ml per litre of water and 3 litres of spray solution per 100 sq.m.
3. Air charge alleyways or gang ways with one of the following chemicals. Malathion 50 EC : 10 ml/litre of water (or) DDVP 76% SC : 7 ml/litre of water. Apply one litre of spray solution for every 270 cu.m. or 10,000 cu. feet. Spray the chemicals on the walls and floors and repeat the treatment based on the extent of flying and crawling insects.
4. Gunny bag impregnation: Empty bags are soaked in 0.1% malathion emulsion for 10 minutes and dried before using for seed storage.

b. Curative treatment

Choose the fumigant and work out the requirement.

i. Aluminium phosphide: The dosage of Aluminium phosphide for cover fumigation is 3 tablets of 3 grams each per tonne of grain and for shed fumigation is 21 tablets of 3 grams each for 28 cu. metres. The period of fumigation is 5 days.

Aluminium phosphide is most commonly used. In case of cover fumigation, mix clay or red earth with water and make it into a paste form and keep it ready for plastering all round the fumigation cover or keep ready sand-snakes. Insert the required number of aluminum phosphide tablets in between the bags in different layers. Cover the bags immediately with fumigation cover Plaster the edges of the cover all round with wet red earth or clay plaster or use sand-snakes to make leak pr*oof*. Keep the bags for a period of *5* - 7 days under

fumigation Remove the mud plaster after specified fumigation period and lift the cover in the corner to allow the residual gas to escape. Allow aeration and lift cover after a few hours. Follow similar steps in case of shed fumigation also.

ii. Methyl bromide (MB): MB which has been widely used in temperate regions of the world has been found unsuitable for Indian conditions as it affected seed germination drastically at temperature above 20°C.

Approved insecticides for the control of stored insect pests as per CIB& RC

S.N.	Insecticides
1	Aluminium Phosphide 56% m/m*
2	Aluminium Phosphide 15% Tablet*
3	Aluminium Phosphide 77.5 % GR*
4	Deltamethrin 2.5% WP
5	Methyl Bromide Technical*
6	Methyl Bromide 98% + Chloropicrin 2% w/w Fumigant
7	Ethlyene Dichloride + carbon tetrachloride (3:1)

*To be used by Govt. approved agencies under expert supervision only whose expertise is approved by the Plant Protection Advisor to Govt. of India.

REFERENCES

Allotey J 1991 Storage insect pests of cereals in small scale farming community and their control. *Insect. Sci. Applic*. **12**: 679-693.

Anand Prakash and Jagadeswari Rao 1995 Insect pest management in stored rice ecosystem. pp 709-736. ***In***: Stored grain ecosystems (Eds) Digvir S J, Noel D G White, and William E Muir. New York: Dekker. 757 pp.

Digvir S J, Noel D G White, and William E Muir eds.1995. Stored grain ecosystems New York: Dekker. 757 pp.

Gahukar R T 1994 Storage of food grains and insect control in developing countries. *Insect. Sci. Applic***.15** (4/5): 383-400.

Mohan. S. 2003 Issues in the management of insects of food grain ***In***: *Proceedings of the National Symposium on Frontier Areas of Entomological Research* pp 423- 438. IARI, New Delhi

Pinglae S.V 1976 Insect and mites attacking food grains ***In***: Handling and storage of food grains: *ICAR*, New Delhi, 127-149.

******

Determination of Seed Viability

Dr. A. k. Verma
Sr. Seed Analyst,
National Seed Research and Training Centre, Varanasi

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 favorable 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. Tetrazoloium 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.

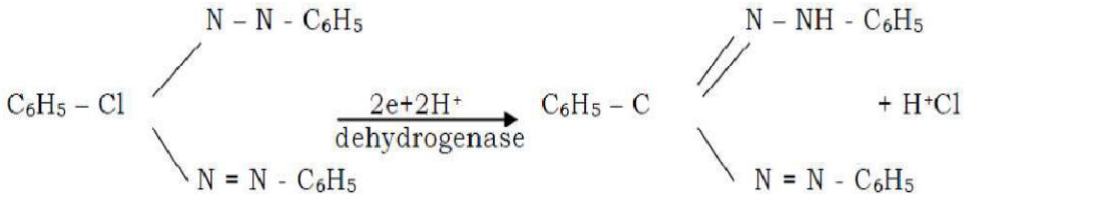
To get 1% of TZ solution, dissolve 1 g of TZ salt in 100 ml of buffer solution. (The one percent solution is used for seeds that are not bisected through the embryo, while the 0.1 percent solution is used for seeds in which the embryo is bisected. Other low concentration such as 0.2 percent and 0.5 percent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



2,3,5 – triphenyl tetrazolium chloride (forms a clear solution in water)

2,3,5 – triphenyl formazan (a red stable, no diffusible substance)

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 colour intensity

a. Dark red –vigours seed.

b. Pink colour -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 coloured embryo and cotyledon.

b. Normal red coloured embryo with only one normal cotyledon.

c. Normal red coloured embryo with half or more than half of both the cotyledons attached to embryo are of red colour.

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 colourless.

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 color 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.

******

Management of Seed Stores

Er. M. K. Vishwakarma
Seed Processing Engineer,
National Seed Research & Training Centre, Varanasi

Seed storage management implies the maintenance of the harvested seed mass in good physical and physiological condition from the time of harvesting upto the time of their replanting. Seed ageing and loss of germination during storage can not 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 vapour seal. Floor insulation is frequently installed in a bed of hot asphalt, which provides a good vapour seal. The types of material used may be fibreglass, 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 vapour 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 vapourising refrigerant; (2) a suction line to convey the refrigerant vapour from the evaporator to the compressor; (3) a compressor to heat and compressor the vapour; (4) a hot gas or discharge line to carry the high-temperature, high-pressure vapour from the compressor to a condensor; (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 tonne of ice in 24 hours is said to have a capacity of one tonne 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 days 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 vapour from the air stream, the remainder is being recharged.

*****

Use of Tolerance Table in Seed Testing

Manimurugan C.
ICAR-Indian Institute of Oilseeds Research, Hyderabad, Telangana

Introduction

The world population is increasing day by day. We need to increase food production to feed future population. Use of quality seed is one of the strategies to increase production and productivity of any crop. Quality seed production involves many steps viz. land selection to avoid volunteer plants, use of certified seeds to confirm the genetic purity of cultivar, keeping isolation distance to avoid genetic contamination, roguing to maintain genetic purity and avoid disease infestation, timely harvesting to avoid deterioration, processing with care to maintain physical purity, seed testing to confirm the quality of seed and seed certification (optional) to ensure seed quality. Seed testing generally carried out with small representative sample of seed lot. The results of replication will vary with one another. If the seed lot is homogeneous, the test result of replications is more or less same. if the seed lot is heterogeneous the results will show huge variation among replications. In this situation, Acceptance of results or retesting the sample will be decided by using tolerance table. Tolerance table used for physical purity analysis and germination test.

History

In 1930 C.W. Leggatt proved that the purity and the gemination data follow the binomial distribution and the foreign seed content (other seed counts) follows the Poisson distribution. He proposed tolerances for purity, germination and foreign seed testing. In 1956, the ISTA Statistical Committee was established in order to develop statistical methods for application to seed testing. S.R. Miles, a member of Statistics Committee developed tolerances for purity, germination and other seed count tests at several significance levels. The current tolerances in the ISTA Rules have drawn from tolerances in Miles's (1963) "Handbook of Tolerances and of Measures of Precision for Seed Testing".

Use of Tolerance Table

Purity tolerances

Tolerances suitable for comparing purity results on duplicate samples from the same submitted sample analysed by the same laboratory are given in table 1. These can be used for any component of the purity test (pure seeds, inert matter, etc.). This table is used by computing the average of the two test results (columns 1 or 2) the appropriate tolerance found in one of columns 3 and 4 for half working samples and in one of columns 5 and 6 for whole working samples, depending on whether the seed type is chaffy or non-chaffy. Tolerances in this table are based on a two-sided test at the 5% significance level (Banyai and Barbas,2002).

Table 1. Tolerances for purity tests on the same submitted sample in the same laboratory (two-way test at 5% significance level)

Average of the two te results		Tolerances for differences between			
50-100%	Less than 50%	Half working samples		Whole working samples	
		Non-chaffy seeds	Chaffy seeds	Non-chaffy seeds	Chaffy seeds
1	2	3	4	5	6
99.95-100.00	0.00-0.04	0.20	0.23	0.1	0.2
99.90-99.94	0.05-0.09	0.33	0.34	0.2	0.2
99.85-99.89	0.10-0.14	0.40	0.42	0.3	0.3
99.80-99.84	0.15-0.19	0.47	0.49	0.3	0.4
99.75-99.79	0.20-0.24	0.51	0.55	0.4	0.4
99.70-99.74	0.25-0.29	0.55	0.59	0.4	0.4
99.65-99.69	0.30-0.34	0.61	0.65	0.4	0.5
99.60-99.64	0.35-0.39	0.65	0.69	0.5	0.5
99.55-99.59	0.40-0.44	0.68	0.74	0.5	0.5
99.50-99.54	0.45-0.49	0.72	0.76	0.5	0.5
99.40-99.49	0.50-0.59	0.76	0.82	0.5	0.6
99.30-99.39	0.60-0.69	0.83	0.89	0.6	0.6
99.20-99.29	0.70-0.79	0.89	0.95	0.6	0.7
99.10-99.19	0.80-0.89	0.95	1.00	0.7	0.7
99.00-99.09	0.90-0.99	1.00	1.06	0.7	0.8
98.75-98.99	1.00-1.24	1.07	1.15	0.8	0.8
98.50-98.74	1.25-1.49	1.19	1.26	0.8	0.9
98.25-98.49	1.50-1.74	1.29	1.37	0.9	1.0
98.00-98.24	1.75-1.99	1.37	1.47	1.0	1.0
97.75-97.99	2.00-2.24	1.44	1.54	1.0	1.1
97.50-97.74	2.25-2.49	1.53	1.63	1.1	1.2
97.25-97.49	2.50-2.74	1.60	1.70	1.1	1.2
97.00-97.24	2.75-2.99	1.67	1.78	1.2	1.3
96.50-96.99	3.00-3.49	1.77	1.88	1.3	1.3
96.00-96.49	3.50-3.99	1.88	1.99	1.3	1.4
95.50-95.99	4.00-4.49	1.99	2.12	1.4	1.5
95.00-95.49	4.50-4.99	2.09	2.22	1.5	1.6
94.00-94.99	5.00-5.99	2.25	2.38	1.6	1.7
93.00-93.99	6.00-6.99	2.43	2.56	1.7	1.8
92.00-92.99	7.00-7.99	2.59	2.73	1.8	1.9
91.00-91.99	8.00-8.99	2.74	2.90	1.9	2.1
90.00-90.99	9.00-9.99	2.88	3.04	2.0	2.2
88.00-89.99	10.00-11.99	3.08	3.25	2.2	2.3
86.00-87.99	12.00-13.99	3.31	3.49	2.3	2.5
84.00-85.99	14.00-15.99	3.52	3.71	2.5	2.6
82.00-83.99	16.00-17.99	3.69	3.90	2.6	2.8

80.00-81.99	18.00-19.99	3.86	4.07	2.7	2.9
78.00-79.99	20.00-21.99	4.00	4.23	2.8	3.0
76.00-77.99	22.00-23.99	4.14	4.37	2.9	3.1
74.00-75.99	24.00-25.99	4.26	4.50	3.0	3.2
72.00-73.99	26.00-27.99	4.37	4.61	3.1	3.3
70.00-71.99	26.00-29.99	4.47	4.71	3.2	3.3
65.00-69.99	30.00-34.99	4.61	4.86	3.3	3.4
60.00-64.99	35.00-39.99	4.77	5.02	3.4	3.6
50.00-59.99	40.00-49.99	4.89	5.16	3.5	3.7

Examples

1. Purity tests on 2 half working samples from the same submitted sample of X (any crop) were made in the same laboratory
   The first result: 97.0%
   The second result: 98.4%
   Average: 97.7%
   Difference: 1.4%
   Tolerated difference: 1.63 for chaffy seeds
   Decision: the two results are **compatible**

2. Purity tests on 2 half working samples from the same submitted sample of X (any crop) were made in the same laboratory
   The first result: 97.0%
   The second result: 98.8%
   Average: 97.9%
   Difference: 1.8%
   Tolerated difference: 1.54 for chaffy seeds
   Decision: the two results are **not compatible**

Separate tolerance tables were given and that that have to utilized for purity tests on two different submitted samples conducted in the same laboratories and two different laboratories.

Tolerances for other seeds by number

Tolerances for the determination of other seeds by number when tests are made on the same or different submitted sample in the same or a different laboratory (Table.2) are suitable to decide if a second estimate is compatible with a labelled analysis. Both sample have to be of approximately the same weight. The table is used by selecting the level equal to the average of the two test results in column 1 to find the maximum tolerated difference in column 2. The tolerances are based on a two-sided test at the 5% significance level (Banyai and Barbas,2002).

Table.2. Tolerances for the determination of other seeds by number

Average of the two test results	Tolerance	Average of the two test results	Tolerance	Average of the two test results	Tolerance
1	2	1	2	1	2
3	5	76-81	25	253-264	45
4	6	82-88	26	265-276	46
5-6	7	89-95	27	277-288	47
7-8	8	96-102	28	289-300	48
9-10	9	103-110	29	301-313	49
11-13	10	111-117	30	314-326	50
14-15	11	118-125	31	327-339	51
16-18	12	126-133	32	340-353	52
19-22	13	134-142	33	354-366	53
23-25	14	143-151	34	367-380	54
26-29	15	152-160	35	381-394	55
30-33	16	161-169	36	395-409	56
34-37	17	170-178	37	410-424	57
38-42	18	179-188	38	425-239	58
43-47	19	189-198	39	440-454	59
48-52	20	199-209	40	455-469	60
53-57	21	210-219	41	470-485	61
58-63	22	220-230	42	486-501	62
64-69	23	231-241	43	502-518	63
70-75	24	242-252	44	519-537	64

Example

1. Two samples of the prescribed weight were tested for the determination of other seeds by number on different submitted samples from the same lot in different laboratories. The aim of the examination is to decide, whether the second test results are compatible with labelled value. Table.2. should be used.
   The first result: 12 seeds of a particular species
   The second result: 27 seeds of the same species
   Average: 19.5
   Differences: 15
   Tolerated difference: 13
   Decision: the two results **cannot be accepted** as compatible

Separate tolerance table was given and that that has to utilized for two samples of the prescribed weight were tested for the determination of other seeds by number on different submitted samples from the same lot. The first estimate was made by the seller and the second test was conducted by an official seed testing station.

Germination tolerances

Tolerances in table 3. for germination tests on two different submitted samples from the same lot on 400 seeds in the same or a different laboratory can be applied to decide whether the results of the second test are below the labelled value. The table gives tolerances for percentages of normal seedlings, abnormal seedling, dead seeds, hard seeds or any combination of these. This table is used by selecting the average (nearest whole number) of the two test results in column 1 or 2 and the maximum tolerated difference is found in column 3. These tolerances are based on a one-sided test at the 5% significance level. This table is suitable to check the validity of the labelled germination percentage value provided by the seller of the seed lot (Banyai and Barbas,2002).

Table.3. Tolerance for gemination tests on two different submitted samples in the same or a different laboratory on 400 seeds

Average percentage		Tolerance	Average percentage		Tolerance
More than 50%	50% or less		More than 50%	50% or less	
1	2	3	1	2	3
99	2	2	82to86	15to19	7
97to98	3to4	3	76to81	20to25	8
94to96	5to7	4	70to75	26to31	9
91to93	8to10	5	60to69	32to41	10
87to90	11to14	6	51to59	42to50	11

Example.

1. Two germination tests were made in two different laboratories each on 400 seeds form different submitted samples from the same lot.
   The first result: 87% declared by the seller
   The second result: 80% obtained by the customer
   Average: 84%
   Difference: 7%
   Tolerated difference: 7%
   Decision: The labelled value can be accepted

Separate tolerance table for 4 replicates of 100 seeds, 2 replicates of 100 seeds, 2 replicates of 50 seeds, 2 tests of 400 seeds, two tests of 200 seeds and 2 tests of 100 seeds were also given International Rules for Seed Testing (ISTA, 2015)

References:

Banyai, J and Barbas, J. (2002). Hand book on statistics in seed testing, ISTA.

ISTA (2015). International Rules for Seed Testing.

*****

Identification of Weed Seeds

S.K.Chakrabarty
Division of Seed Science and Technology, ICAR-IARI,New Delhi-110012

Increase in seed trade has promoted increased transfer of seeds of crop varieties from one region and country to other. Association of seeds of other varieties and weeds in a seed lot is a well known. It not only reduces the quality of seed but its nature. Weeds disseminate to new habitat through the crop seeds. It is also common that weeds produce enormous number of seeds every generation.

Before seeds are marketed within or to outside the country, its testing is mandatory. In seed testing, it is essential to test the seed samples for physical purity including the presence of weed seeds. Through seed testing, contamination by weeds and other crop seeds are avoided. In modern seed testing, authentic identification of weed seeds in crop varieties is vital.

Government of India took several steps including framing and implementing seed legislations for regulation of quality of being used for further multiplication nd cultivation. The Seeds Act was enacted in 1966 and ensuing Seed Rules framed under The Seeds Act, were notified in 1968 and since then several amendments have been carried out in Seeds Act and Seed Rules from time to time for regulation of seed. Government of India has notified Indian Minimum Seed Standards [IMSCS] for ensuring the seed quality *viz*., Genetic purity, physical purity, germination, moisture, and seed health. The physical purity from seed testing point of view refers to physical or mechanical purity of seed lot. The results of purity analysis reflect the physical quality status of seed lot. These results are of great significance to seed processors and seed certification or seed law enforcement agencies (to judge whether the seed lot confirms to the prescribed standards or not). As per Indian Minimum Seed Certification Standards the physical purity of the seed lot consists of (i) pure seed (%) (ii) maximum inert matter (%) (iii) maximum other crop seeds (no/kg) and (iv) maximum weed seeds (no/kg) including maximum objectionable weed seeds (no/kg). As per ISTA Rules the working sample is separated into three components i.e., pure seeds, other seed, and inert matter. The percentage of each part is determined by weight. Other seeds are undesirable because:

- They are a source of weed introduction to new areas
- Once established, weeds are difficult to eradicate
- High contamination will lower purity content
- Risk of rejection due to lower seed standards

Need for accurate identification of weed seeds

Weed seeds as concomitant admixtures always affect physical purity of seed lots.Since the inception of seed testing, correct seed identification has been the basis for purity analysis. Seed collection, seed illustrations and descriptions of seed morphology have been valuable tools in the identification of unknown seeds. The experiences in the field of establishment and maintenance of seed collections were presented at International Seed Testing Association [ISTA] workshops in Wageningen [Jensen, 1979d] and Budapest [Jensen, 2000].

Accurate identification of seeds, both crop seeds and weed seed contaminant is necessary for correct labeling of seed moving in commercial channels. There are many other groups of plants in which seeds of one species may closely resemble to those of another species, some cases those may be undesirable or noxious weed seed, so seed analyst must be able to analyze and evaluate these structures. Internationally weed seed identification and its application in field of seed science and technology is administered by ISTA. In ISTA, identification of weed seeds, developing and adopting tools for physical purity analysis of seed lots is governed by ISTA Purity Committee. Working group on seed identification was initiated in the year 1977. It consisted of collection and identification of crop and weed species through seed morphology.Many working programmes were also initiated within the purity committee for preparation of universal list of weed species, in 2004 and 2006 two more working programmes were also initiated for identification of crop and weed seeds and documentation of digital images of weed species to aid identification of seeds. Identification of weed seeds in the seed lot requires a detailed knowledge of gross seed morphology of disseminule produced by plants. Identification of weed seeds is extremely important for seed quality analysis for the issue of Orange International seed lot certificate and Blue International seed sample certificate and also for routine seed quality analysis, seed certification etc., and for ISTA accreditation of seed testing laboratory.

Seed identification

It is sometime a difficult job to identify a seed of a crop or weed species. An in-depth knowledge of botany of a plant as well as its seed is necessary, for correct identification of a particular species. In systematic botany or taxonomy, the closely related or similar type of plants are grouped into a single category. These groups are: family, genus, species etc. In seed identification the particular seed in question must be identified up to the species level.

The seed, a mature ovule consists of an embryo a protective covering and stored food as endosperm. The identification of seed is usually by comparison, comparing the seeds with a mental image of what something should be, with specimens in a reference collection or with illustration of seeds. In most cases, the useful clues for the identification of seeds come from the following characters:

1. The size, shape and colour of seeds
2. The nature, arrangement and pattern of markings that is lines, ridges, pits, projection on the seed surface
3. The shape and position of the attachment scar
4. The presence of wings, hairs or scales, spines etc
5. The internal structure, position and size of the embryo, presence or absence of the endosperm

Seed keys are developed based on characters pertaining to family, genus, and species. Once the seed is characterized for a particular family, identification of the seed could easily be made by studying the above-mentioned seed characters. Quite often it is difficult to identify the seeds as such. In such situation, growing it to a plant could aid in identification of seed. The original seed sample of the species is always helpful in identification of unknown unconventional crop and weed seeds.

Seed characteristics of some common families

1. ***Gramineae:*** seed unit is a caryopsis, a fertile floret a spikelet or a spike. The embryo lies on outside of the endosperm and visible near the base of caryopsis on dorsal side.
2. ***Leguminosae:*** Seeds vary greatly in size, shape and surface characters. The fruit may be one seeded in several-seeded pod.
    - ***a) Mimosoideae and Caesalpinodeae***
        - i) The seeds are elongate broad and flattened, the two faces being plane or only rounded, colour is varied from black to white and yellow
        - ii) The hilum is very small, unspecialized and located at one end of the seed.
    - ***b) Papilinoideae***
        - i) The seeds vary greatly in size, shape, colour and location of hilum and chalaza.
        - ii) In hilum, there is a fine longitudinal groove or slit down the middle. The area may be minute, as in some of the clovers, or may be large enough to be seen without magnification as in vetch.
        - iii) In some species the hilum is obscured by a persisting layer of corky tissue, as in cowpea and beans.
3. ***Cruciferae:*** The seeds unit may be a true seed, in indehiscent pod or a segment of a pod.
    - a) The seeds are mostly spherical, or sometimes slightly flattened.
    - b) The surface has reticulum or netting or lines or ridges.
    - c) The seed surface is covered with microscopic pits. These pits are usually covered with a whitish film, giving the appearance of white spots on the surface.

4.***Polygonaceae:*** The fruit or so-called seed is an achene which is three angled orflattened.The outer hull (pericarp) is hard, brown and glossy.

5.***Chenopodiaceae:*** The seeds are flattened, circular or obovate in shape.
    - a) The embryo is either in the form of a ring or horseshoe.

6.***Caryophyllaceae:*** The seeds are black or brown, thick and flattened.
    - a) The scar lying on the edge.
    - b) The surface is roughened by tubercles of various types which are arranged in definite pattern.

7. ***Euphorbiaceae:*** Seeds vary greatly in size, shape and surface configuration.
    - a) The scar is a flattened area at the base. In some species the scar is obscured by caruncle (whitish corky outgrowth).
    - b) Distinctive feature of the seed in this family is the presence of prominent raphe.
8. ***Solanaceae:*** The seeds are orbicular, oval or ovate. They are more or less flattened and may be thick or thin.
    1. The embryo is curved with an abundant endosperm.
    2. The seed surface may be smooth, or variously configured with a reticulum, broken lines or pits.

9. ***Compositae:*** The seed unit is an achene, which is an indehiscent, one seeded fruit. The top of the achene is usually depressed. In many species there is a fringe of fine bristles or scales around the outer rim.

Characteristic of some common weeds

Family: *Caryophyllaceae*

Spergula arvensis: The seed 1-15 mm diameter lens shaped, dull black, thin, flattish with winged. Embryo, Linear, 'U' shape without endosperm.

Family: *Chenopodiaceae*

Chenopodium album (Bathva): The seed is circular, flat, and round; diameter 1-11/2mm, colourblack, smooth and shiny surface.

Chenopodium murale (Bathva): Similar to *C. album* but slightly bigger in size and dull in appearance.

Family:*Convolvulaceae*

Convolvulus arvensis (Field weed): The seed colour, dull grayish brown, length, 4 to 4½ mm; surface roughened with fine tubercles or short wavy lines. Back side convex and lateral plane, scar: inverted 'U' shape and at right angles to the seed's long axis.

Ipomea hederacea: The seed diverse in shape (trigonous wedge, two inner faces are equal): size (lanceolate, ovoid to globose surface; smooth and colour: brown black. Scar: horseshoe shape and usually parallel to long axis.

Family:*Poaceae*

Avenafatua (Wild oat): The seed consists of mature floret, narrowly cylindrical, tapering at apex, bears a twisted and bent dorsal awn, ventral side flat with fine grooves; colour: grey, brown or black, yellow to white.

Panicumspecies(grasses): The seed unit consists of one seeded spikelet. The grain surrounded by glumes (thin and papery). Lemma and Palea (hard, smooth and shiny, size: 1½ to 2¾ mm usually lance shape).

Setariaetalica: The seed unit consists of one seeded spikelet. The grain surrounded by glumes (thin, papery, and smooth). Lemma and Palea (hard, smooth, and shiny)

Family:*Liliaceae*

Asphodelustennuifolius (wild onion): The seed 1¼ long, flattened elliptical three angled (sharp) acute and black (crustaceous) testa.

Family: *Papaeraceae*

Fumaria parviflora: Fruit very small, globose, one seeded, indehiscent nutlet, rugose when dry and rounded at the top with two pits, color usually green.

Family: *Papillionaceae*

Medicago sativa (lucerne): The seed roughly oval (scar lies in broad indentation near one end or kidney shape twisted the alongaxes (scar lies in middle of a distinct notch). Colour greenish yellow or light brown, length 1½ mm and width 2½ mm to 3mm.
Melilotus alba (white sweet clover): The seed is identified by size (bigger length about 2½ mm and width 1½ mm), shape oblong to oval and translucent in appearance), and colour (golden yellow to light brown). Scar lies in shallow indentation near top.

Family: *Polygonaceae*
Rumexsp (wild spinach): Seed three sided acute as both ends, brown, spinning segments if present with long, fine teeth on the margins.

Seed Standards [weed seeds (max.)] as per Indian Minimum Seed Certification Standards in field crops:
Referred table clearly depicts role of correct identification of weed seeds for certification vis-à-vis quality assurance purpose.

Crop	Total weed seeds		Objectionable weed seeds		Remarks (Objectionable weed seeds)
	Foundation	Certified	Foundation	Certified	
Barley	10/kg	20/kg			
Paddy	10/kg	20/kg	2/kg	5/kg	Wild Rice *(Oryza sativa* L. var. *fatua*Prain)
Wheat	10/kg	20/kg	2/kg	5/kg	*Convolvulus arvensis* *Phalaris minor*
Maize	None	None	-	-	-
Sorghum	5/kg	10/kg	-	-	-
Pearl millet	10/kg	20/kg	-	-	-
Chickpea	None	None	-	-	-
Black gram	5/kg	10/kg	-	-	-
Green gram	5/kg	10/kg	-	-	-
Pigeonpea	5/kg	10/kg	-	-	-
Castor	None	None	-	-	-
Groundnut	None	None	-	-	-
Mustard	10/kg	20/kg	5/kg	10/kg	*Argemone mexicana*
Safflower	5/kg	10/kg	None	None	*Carthamusoxyacantha*
Soybean	5/kg	10/kg	-	-	-
Sunflower	5/kg	10/kg	None	None	*Orobanchecumana*
Cotton	5/kg	10/kg	-	-	-
Berseem	10/kg	20/kg	5/kg	10/kg	*Chicoriumintybus*
Lucerne	10/kg	20/kg	5/kg	10/kg	*Cuscuta spp.*
Napier grass	-	-	None	None	*Cirsium arvense*

(slips)					*Cuscuta spp. Sorghum halepense Agropyronrepens Convolvulus arvensis*
Oats	10/kg	20/kg	2/kg	5/kg	*Avenafatua*

Tools and resources for seed identification

An old Chinese proverb "One picture is worth of thousand words" is especially applicable to the identification of seeds. Enormous literature in the form of atlas, compendium and handbook are available for identification enumerating species of temperate region, but very miniscule information in this regard available for tropical species particular to Indian sub-continent. Digital weed seed atlas consisting of digital seed images and descriptions of species based on morphological keys will effectively supplement seed analyst for easy identification and significantly improves the efficiency of the seed testing laboratories. Seeds of some kind of plants are sufficiently distinctive that they are not easily confused with those of other kinds and their identification poses no problems. There are many groups of plant, in which seeds of one species may closely resemble with seeds of other species. In some cases, one of these may be a crop plant and the other an undesirable or designated weed species. The seed analyst must be able to analyze and evaluate the structures of such seeds in relation to those of other similar species; in such scenario digital weed seed atlas would become handy.

A guide with colour photos of 200 species including agricultural, horticultural and weed species published by National Institute of Agricultural Botany (NIAB), United Kingdom [Jones et al. 2004], a descriptive and illustrated seed-book on 175 weeds were worked out by French Group for Study and Control of Varieties and Seeds (GEVES), France in 2004. More than 1400 images of seeds and fruits have been made available online by the efforts of Dr. Arnold Larsen, Colorado state university, USA; the website [www.seedimages.com] includes colour images of seeds as well as descriptions and keys for identification of described species. Professor Miller McDonald and his staff at Ohio State University, USA, have placed number of seed images of cultivated and weed species with useful basic and advanced seed identification quiz. Dr. John H. Wiersema and his colleagues at Germplasm Resource Information Network (GRIN), United States Department of Agriculture/Agricultural Research Service have added over 3,800 seed/fruit or embryo images or drawings to GRIN, with plans to add another 5,000 in the future [Jensen, 2008]. A quick start weed guide (expert system) with seed identification keys which makes use of 21 externally visible characters (e.g., colour, shape, size and texture) using a computerized database has been developed to identify weed seeds prohibited/restricted by the Australian Quarantine and Inspection Service [Gupta et al., 2005]. Weed seed atlas was compiled depicting digital images of common agricultural weed seeds of USA with descriptions of seed characteristics for its effective usage in seed quality testing laboratories [Schuler, 2009]. In India weed seeds have been collected from crop fields, seed processing plants, marketplaces etc., characterized and identified (Chakrabarty and Tomar, 2005).

1. **Seed herbarium or reference collection**

   A seed herbarium is a standardized collection of seed specimens that has a practical value to the seed analyst in aiding identification and for visual comparison of seeds.These seeds are collected by naturalists, classified, and stored.The easiest means to arrange the seed herbarium is to place it in alphabetical order. Families are arranged alphabetically. Genera within the family are then placed in alphabetical order. And then the species within each genera arranged by the alphabet. This arrangement makes it easy to retrieve specimens but gives no clue to the relationship between the specimen and other species, genera, or families

2. **ISTA reference**
   - ISTA International Rules for Seed Testing
     https://www.seedtest.org/en/international-rules-for-seed-testing-_content---1--1083.html
   - ISTA List of Stabilized Plant Names (7th Edition)
     https://www.seedtest.org/upload/cms/user/ISTAListofStabilizedPlantNamesed.75.pdf
   - ISAT Universal List
     https://www.seedtest.org/en/universal-list-_content---1--1446.html
   - ISTA Handbook on Pure Seed Definition
     https://www.seedtest.org/en/productdetail---------137.html

3. **Seed reference books**
   - Handbook on Tropical Species
   - NIAB Seed Identification Handbook
   - Identification of Crop and Weed Seeds
   - Illustrated Taxonomy Manual of Weed Seed
   - The Encyclopedia of Arable Weeds
   - The illustrated guide to Weed Seeds of New Zealand
   - The Digital Seed Atlas of the Netherlands
   - A Manual for the Identification of Plant Seeds and Fruits
   - Seed Purity and Taxonomy

4. **Online resources**
   - USDA National Germplasm System
     https://npgsweb.ars-grin.gov/gringlobal/taxon/abouttaxonomy
   - Seed Identification Guide (SIG)
     https://www.idseed.org/seedidguide/
   - Colorado State University (requires paid subscription)
     https://www.seedimages.com/Default.aspx
   - The OHIO State University
     https://www.oardc.ohio-state.edu/seedid/
   - USDA Seed Images

https://frontrangeseedanalysts.weebly.com/usda-plates.html

- Flower Seed Images
  https://frontrangeseedanalysts.weebly.com/flower-seed-images-frsa-1995.html
- Digital Plant Atlas
  https://www.plantatlas.eu/

******

Advancement in Seed Health Testing

B.K. Sarma
Department of Mycology and Plant Pathology, Institute of Agricultural Sciences,
Banaras Hindu University, Varanasi

Seed is the basic unit of production for the world's food crop. In recent years seed has become an international commodity used to exchange germplasm around the world. Seed is, however, also an efficient means of introducing plant pathogens into a new area as well as providing a means of their survival from one cropping season to another (Walcott et al., 2003). Great Famine, also called Irish Potato Famine, famine that occurred in Ireland in 1845–49 when the potatocrop failed in successive years. The crop failures were caused by late blight, a disease that destroys both the leaves and the edibleroots, or tubers, of the potato plant. The causative agent of late blight is the water mold*Phytophthorainfestans*which is also transmitted via potato tubers used as seed. As a direct consequence of the famine, Ireland's population of almost 8.4 million in 1844 had fallen to 6.6 million by 1851. About one million people died from starvation or from typhus and other famine-related diseases. The number of Irish who emigrated during the famine may have reached two million. Seed testing is thus required to establish whether seed is infected. Seed health tests are currently available to detect the most important seed-borne pathogens of crops. Only seed that is pathogen-free should be used for sowing. Testing seed before sowing will identify potential disease problems and allow steps to be taken to reduce the disease risk. Laboratory testing is usually required, as infected seed may have no visible disease symptoms. Seed health testing is thus routinely carried out in most countries for domestic seed certification, quality assessment and plant quarantine (FAO, 2010). Seed health testing is an integral for all seed companies in disease risk management (ISF, 2010). Seed health is a well-recognized factor in the modern agricultural science for desired plant population and good harvest (Rahman *et al*., 2008). Seedborne pathogens are a continuing problem and may even be responsible for the re-emergence of diseases of the past as well as the introduction of diseases into new areas (Gitaitis and Walcott, 2007). Seedborne pathogens present a serious threat to seedling establishment (Walcott, 2003). In today's global economy, seed accounts more than ever for the movement of plant pathogens across vast distances, natural barriers, and political borders (Gitaitis and Walcott, 2007). The quality of planted seeds has a critical influence on the ability of crops to become established and to realize their full potential of yield and value (McGee, 1995).

Seed-borne fungi are one of the most important biotic constrains in seed production worldwide. They are responsible for both pre and post-emergence death of grains, affect seedling vigor, and thus cause some reduction in germination and also variation in plant morphology (Van Du *et al*., 2001; Rajput *et a*l., 2005; Niaz and Dawar, 2009). The seedborne pathogens may result in loss in germination, discolouralion and shrivelling, development of plant diseases, distribution of pathogen to new areas, introduction of new strains or physiologic races of the pathogen along with new germplasm from other countries and toxin production in infected seed (Agarwal and Gaur, Undated). Fungi outnumber all other

types of pathogens that attack plants and cause a very serious economic impact on agricultural production due to their ability to induce diseases of cultivated crops that result in important yield losses (Paplomatas, 2006).

There are three primary organizations that publish standardized seed health test methods for use in international trade. These are International Seed Testing Association (ISTA), International Seed Health Initiative (ISHI), and in the United States, the National Seed Health System (NSHS) (Munkvold, 2009). Two of the most important concepts in seed health testing are sensitivity and selectivity, which are inextricably linked. For example, increasing the selectivity of semiselective media may decrease the recovery efficiency of all or some strains of the target organism. In contrast, increasing selectivity may reduce the number of nontarget organisms that act as competitors and/or inhibitors that interfere with the assay, and thus increase the detection sensitivity (Roumagnace*et al*., 2000; Toussaint *et al*., 2001; Wydra*et al*., 2004). A semiselective 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 (Hajihasani*et al*., 2012; Tsedaley 2015).

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 phytosanitary 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 seedborne 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 may be 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).

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. Seed borne pathogens results in seed rots, seedling decay, pre and post emergence mortalities, abnormalities, discoloration, reduced seed size and shrivelling 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 loos of human life and rupees. Late blight of potato caused by *Phytopthorainfestans*was responsible for Irish famine during 1845. Nearly one million people died and almost an equal number of people emigrated from Ireland. Brown spot of rice caused by the seed borne *Helmenthosporiumoryzae* was responsible for Bengal Famine during 1942. More than 2.1 million people died. The loss by downy mildew of pearl millet in Rajasthan in 1962-64 was estimated to be 20 million rupees. Occurrence of tobacco bud blight in soybean caused 66.43% yield loss. AICRP on millet showed an average annual loss of about 30% in high yielding varieties equivalent to 270 million $ annually. Cause economic losses in cultivated crops worldwide.

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	*Alterniaradicina* *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	*Phytophthora parasitica* *Pythium aphanidermatum* *Alternaria solani* *Phytophthora infestans*
Wheat	Loost smut Karnal smut Flag smut	*Ustilago segetum var. tritci* *Neovossiaindica* *Urocystisagropyri*
Rice	Bunt False Smut Stack burn	*Neovossia horrid* *Ustilaginoideavirens* *Pyriculariaoryzae* *Trichoconiellapadwickii*
Maize	Black kernel rot Cob rot Southern leaf blight	*Botryodiplodiatheobromae* *Fusarium moniliformae* *Dreschlera maydis*
Pearl millet	Downy mildew Smut	*Sclerosporagraminicola* *Tolyposporiumpenicillariae*
Sorghum	Anthracnose	*Colletotrichum graminicola*

	Kernel or grain smut Downy mildew	*Sphacelothecasorghi* *Peronosclerosporasorghi*
Cotton	Anthracnose Wilt Alternaria blight	*Colletotrichum indicum* *F.oxysporumf.sp.vasinfectum* *Alternaria macrospora*
Sugarcane	Red rot Wilt Pineapple Disease Smut Mosaic(s)	*Colletotrichum falcatum* *Fusarium moniliforme* *Ceratocystis paradoxa* *Ustilagoscitaminea* *Virus*
Groundnut	Charcoal rot Crown rot Yellow mould/Aflatoxin	*Macrophominaphaseolina* *Aspergillus niger* *Aspergillus flavus*
Sunflower	Alternaria blight Downy mildew Charcoal rot	*Alternaria helianthi* *Plasmoparahalstedii* *Macrophominaphaseolina*
Soybean	Anthracnose Pod & stem blight Purple seed stain	*Colletotrichum dematium* *Phomopsis sojae* *Cercosporakikuchii*
Chickpea	Ascochyta blight Wilt Graymold Alternaria blight	*Ascochytarabiei* *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

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 areBrassica 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, the Orange alert diseases includeCarrot Fungal Blights caused by Alternaria blight (*Alternariadauci*), Cercospora blight (*Cercosporacarotae*); Tomato bacterial diseases caused by Bacterial canker (*Corynebacterium michiganense* pv. *michiganense*), Bacterial Spot (*Xanthomonascampesiris* pv. *vesicatoria*), Bacterial Speck (*Pseudomonassyringae*); Onion white rot fungus (*Sclerotiniasclerotiorum*).

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.

References:

Walcott RR, Gitaitis RD, Castro AC. 2003. Role of blossoms in watermelon seed infestation by *Acidovoraxavenae* subsp. *citrulli*. Phytopathology 93:528–34.

Gitaitis R. and Walcott R., 2007. The Epidemiology and Management of Seedborne Bacterial Diseases. Annual Review of Phytopathology 45:371–97.

McGee D.C., 1995. Approach to disease management through seed technology. Annual Review of Phytopathology 33:445-466.

Tsedaley B. 2015. Review on Seed Health Tests and Detection Methods of Seed borne Diseases. Journal of Biology, Agriculture and Healthcare 5(5):176-814.

******

Latest seed testing equipments used in seed testing and their maintenance

Manimurugan C.
Senior Scientist, ICAR-Indian Institute of Oilseeds Research, Hyderabad, Telangana

Quality seed availability to farmers is very important to ensure the higher production and productivity of any crops and self sustainably in food production. Seed testing laboratories in India confirm quality of seeds distributed to farmers by conducting various tests. Advancement in seed testing needs to be incorporated in seed testing laboratories for fast and accurate seed testing of increasing demand in seed industries.

Equipment / Apparatus for purity analysis

Seed scanner, digital microscope camera, LED transmitted light mounted microscope, blower with monitoring and calibration facility, continuously flowing blower and ergo vision 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.

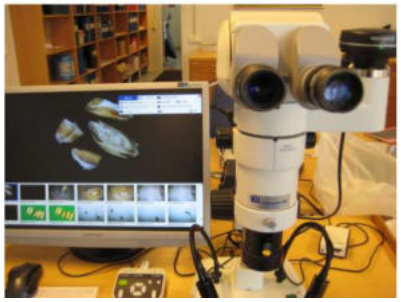


Fig.2 Digital microscope camera

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 transmitted light mounted microscope
(b). As seen in microscope with light above
(c). As seen in microscope with LED transmitted light from below

a. Light weight, immature and diseased seed

b. Medium weight seed

c. Heavy weight selected seed

Fig.4 Continuous flowing blower

Continuous Flowing blower

Continuously flowing blower (fig.4) can separate the sample in to three fractions (fig.5). It is designed with built in anemometer. Air velocity can be calibrated. Any size of seed and large sample can be analysed.

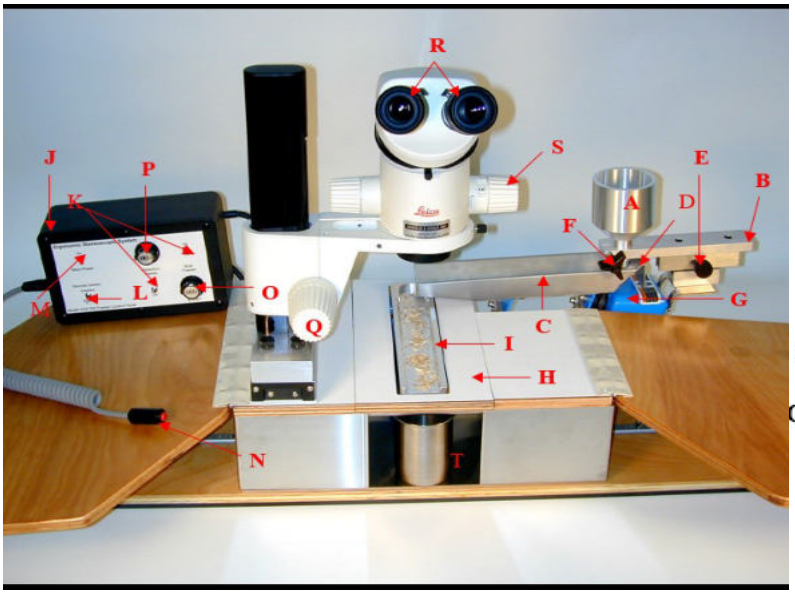
Fig.5. Clover seeds separated by Continuous flowing blower

Ergovision 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
T. Collection Cup

Full HD Digital Microscope for Other seed testing

Microscope attached with camera and computer system make it easier to analysis the small weed seeds and other impurities during physical purity analysis of seeds. The data and photos can be saved and used for further references.

Video meter

Nowadays purity analysis is possible with purity instruments using imaging and Artificial intelligence (AI).

Fig 6. Videometer

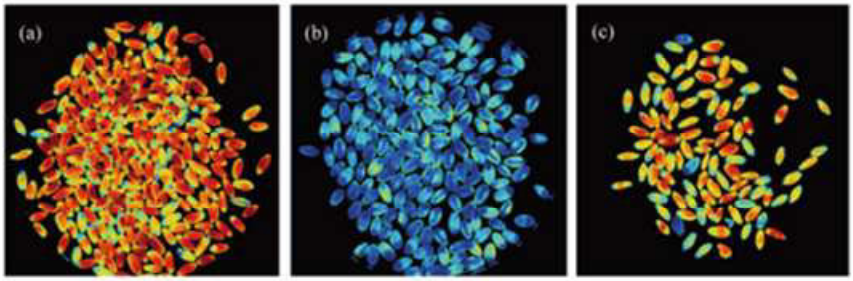


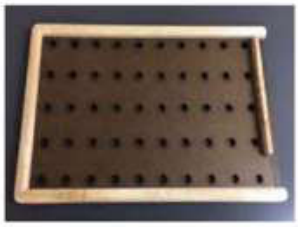
Fig 7. Examples of images generated using the Videometer Lab 2 system (a) 100% T. durum wheat grains; (b) 100% *T. Aestivum* wheat grains; and (c) 10% adultration of *T. durum* wheat grains with *T. aestivum* wheat grains

Imaging techniques, NIR spectroscopy, or precise remote sensors could be combined with conventional methods for better results. A combination of spectral imaging, thermal imaging, fluorescence imaging, X-ray imaging, and magnetic resonance imaging offers reliable alternatives to the traditional methods (Li et al. 2014). Videometer (Fig. 6, (Source: https://analytik.co.uk/)), could be used in purity analysis to find out ODVs (Wilkes et al. 2016), inert matter, and weed seeds (Fig. 7).

Counting Devices

Automatic seed counter, seed counting boards and vacuum seed counters are used in seed testing laboratories to keep a greater number of samples for germination testing in shorter period. Possibilities of cross contamination can be avoided by cleaning equipment after handling each sample.

Automatic seed counter



Seed counting boards

Vacuum seed counter

Advancement in germination testing

In this information technology (IT) and globalisation era seed analyst and researchers are trying to integrate the IT advancement in seed biological research and developing non-destructive methods to assess the physiological and genetic quality of seed. Apart from conventional testing methods, some of the advanced supportive tests will bring accuracy in germination testing result.

Digital imaging information technology

Digital imaging information technology can be effectively applied in germination testing to fulfill the requirements of our modern strategy of "precision agriculture" (Dell' Aquila, 2009). Automated computer methods are utilized to speed up the seed quality assessment. Interest is increasing in the development of machine vision systems to replace human visual inspection, usually employed in germination tests under the rules of the ISTA (2005) and AOSA (2000).

Germination of seeds of *Brassica* genera are most studied under image analysis system because of its morphology and shape. Imbibition and subsequent germination of seed can be effectively monitored by machine vision system. The machine vision prototype (fig.8) consists of a thermostatic chamber at 25 °C. It includes a color Charged-Coupled Device (CCD) camera (Micropublisher 3.3M pixels, Q imaging, Canada), a timer-dependent lighting system, and a holder for a Petri dish containing polymerized agarose where a sample of a maximum of twelve Brassica seeds could be placed. Alternatively, agarose was substituted with highly concentrated NaCl for salt stress imbibition trials (Dell' Aquila, 2003),or deferent temperature regimes were used to evaluate their effects on germination performance (Dell' Aquila, 2005). The computer unit was standardized using a CCD camera with a 55-mm telecentric lens or a flat-bed scanner (Sharp mod. JX-330, Japan) for image capturing, a commercial imaging board, and a high-power personal computer running MS® Windows XP. The most recent version of the commercial software package Image Pro-Plus™ (IPP; Media Cybernetics, USA), or alternatively the open-source freeware software Image (http://rsb.info.nih.gov/ij/) were used for digital image processing. Time-lapse seed images were captured every hour in an automated way and

saved on the hard disk in the most common graphic for mats with a full color option. Image segmentation was carried out to streamline the process of object contour identification and to define the silhouette of the seeds.

Fig.8. The machine vision prototype

Image segmentation is the process of grouping pixels of a given image into homogeneous regions with respect to the contour of the object and the surrounding background, i.e. the inert support where seeds were placed. multi-thresholding algorithm was used, which is able to differentiate the 2-D color pair histograms(i.e. RG, RB, GB segmentation maps), giving a comprehensive segmentation map (Kurugollu*et al.,* 2001).A macro was created with Image-pro's macro language (IP Basic, Media Cybernetics, USA) for the conversion of pixels into millimeters, object number counting, image analysis parameter measuring and data transfer to a MS® Excel worksheet (Dell' Aquila, 2009).

A more sophisticated image acquisition system was developed to capture images of deferent trays, containing plugs in which lettuce, cauliflower and tomato seeds were grown for subsequent transplanting (Ureña*etal*. 2001). Once the CCD camera was positioned over a given tray, a label placed on the tray surface containing the serial number in bar code form was read. Then, an image of the tray was obtained, allowing the examination of the cells to search for germinated seeds; the degree of seedling development was classified using fuzzy logic, and processed data on germination percentage and length of each seedling represented indices of speed of germination. More recently, Ducournau*et al*. (2004, 2005) elaborated new algorithms based on the idea that the emergence of a radicle tip at a defined time results in a modification of the binary images. The system was tested to study germination of sunflower (*Helianthusannuus*L.) seeds, and detailed germination curves were obtained, allowing a perfect fit in a probit model (Ellis and Roberts, 1981).

The following information flow are generated by the computer imaging process (Dell' Aquila, 2009).

1) Sequence of germination process can be recorded as Joint Photographic Experts Group (JPEG) or Tagged Image File (TIF) format and this can be converted in to Audio Live Interleave (AVI) format and utilized for educational purpose.
2) Different parameters (area, perimeter, length, width, roundness and surface colour space value) of single seed in a population are recorded and different complex parameters are generated with help of software and correlated with germination process.

3) Recent development in software and hardware favour statistical and graphical processing of large quantity of data. So that we can compare different seeds of a homogeneous population, seed lots of different variety, aged and fresh seed lots with respect to their germinability.
4) By measuring the protrusion of the radicle tip and its growth, the vigour of the seed also assessed with in shorter period of time.

The creation of an extensive database could allow matching of deferent bio-morphological characteristics of a large number of seed species to improve genetic purity analysis, taxonomy screening, germination and vigour prediction.

Red-Green-Blue color space evaluation in seed digital images

Physiological studies have demonstrated that there are only three color sensors utilized by human visual recognition, which are associated with long (Red, R), medium (Green, G) and short (Blue, B) wavelengths of light (Fairchild,1998). RGB color order system used to study color features of seed digital images. This is generally altered by surface texture, lighting, shading effects and viewing conditions. By automated image analysis all these factors must be considered in the characterization of a colored image of a seed through a series of color histograms. RGB color components on lentil seed samples stored under different deteriorating conditions were analysed. RGB color primaries can be used as a non-destructive marker in classifying seeds with deferent viability levels within a seed population (Dell' Aquila, 2006).

LED-Induced Hyper spectral Reflectance Imaging

Mo, C *et al* (2013) developed a viability evaluation method for cucumber (*Cucumis sativus*) seed using hyper spectral reflectance imaging (fig 9).

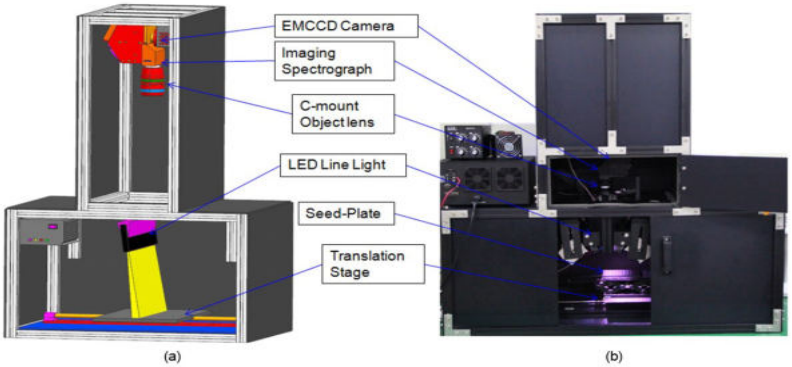


Fig.9. (a) Schematic diagram and (b) photo of the hyperspectral reflectance imaging system

Reflectance spectra of cucumber seeds in the 400 to 1000 nm range were collected from hyper spectral reflectance images obtained using blue, green, and red LED illumination. A partial least squares–discriminant analysis (PLS-DA) was developed to predict viable and non-viable seeds. Various ranges of spectra induced by four types of LEDs (Blue, Green, Red, and RGB) were investigated to develop the classification models.

PLS-DA models for spectra in the 600 to 700 nm range showed 98.5% discrimination accuracy for both viable and non-viable seeds. Hyper spectral reflectance images made using LED light can be used to select high quality cucumber seed.

Q2 technology

The Q2 equipment provides a quick and precise measurement of the oxygen consumption of each individual seed (fig 10). The Q2 technology can give insight into the germination speed and uniformity of a specific seed-lot. Q2 tests are non-destructive, which allows for further testing of the seeds in more conventional ways **(astec-global)**.

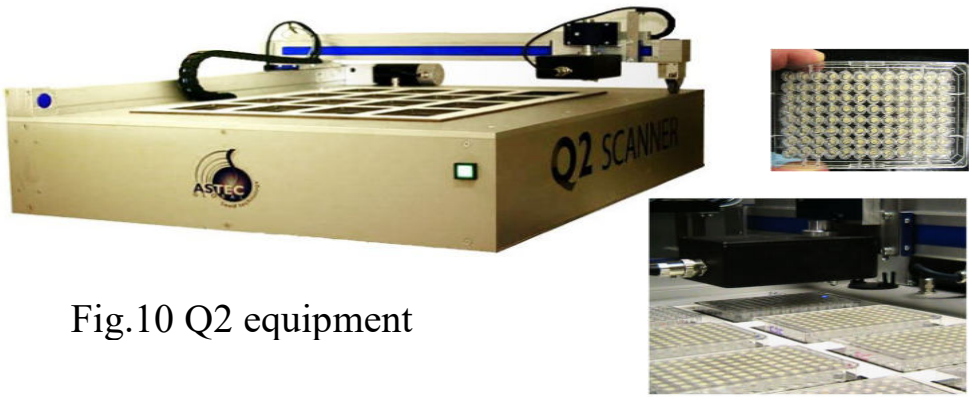


Fig.10 Q2 equipment

Some major applications of Q2 technology

Fast germination measurement tool
Complete picture of seed vigor
Easy control over seed processes
Predicting the feasibility of priming methods
Help in breeding programs
Germination results in 24-48 hours
Easy and fast control of aging seed inventory
Checking bacteria and fungi contamination levels
Homogeneity tests

Dynamic speckle or biospeckle technology

More recently, a new technology has been developed known as dynamic speckle, or biospeckle, an optical phenomenon produced when living materials, such as biological tissues, are illuminated by laser light (fig 11). The term speckle refers to a random granular pattern which can be observed when a highly coherent light beam is diffusely reflected on a surface with a complicated structure. Features of seed tissue images, acquired by a CCD camera, can be amplified and assessed by their speckle activity by appropriate algorithms (Braga*et al.*,2003).

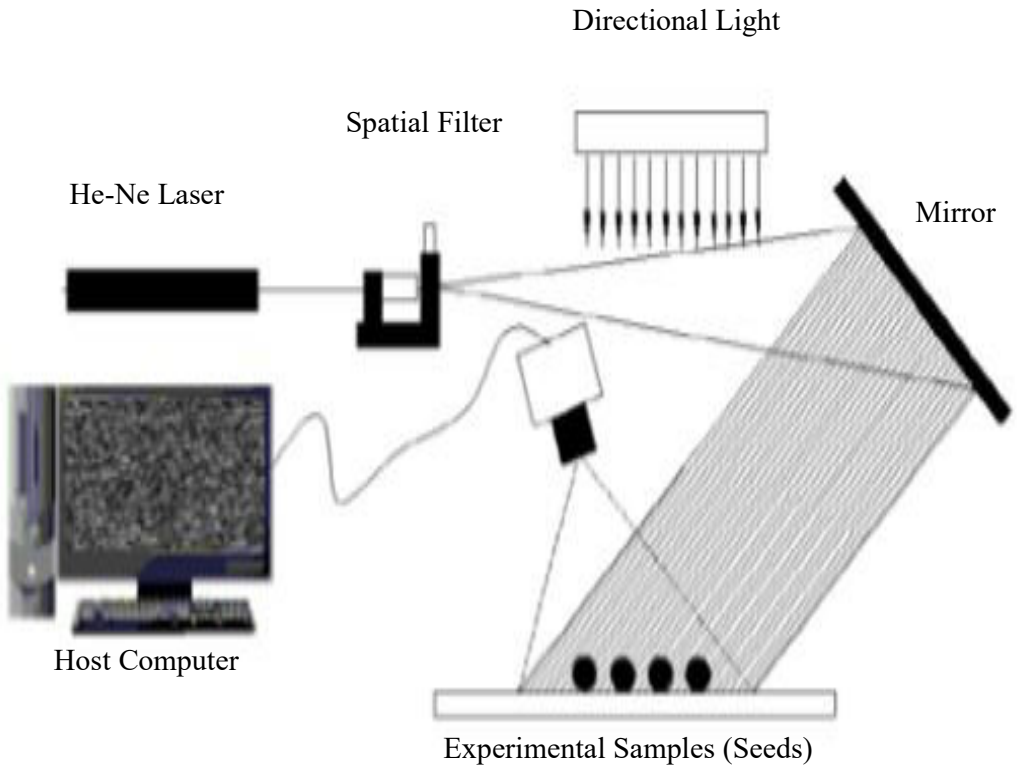


Fig.11. Illumination and image capture experimental system in Dynamic speckle, or biospeckle technique.

The biospeckle technique has proved to be a potential non- destructive methodology to assess bean (*Phaseolus vulgaris* L.) seed viability, even if water content in the seeds can effects measurements. The technique has also been applied to detect the presence of fungi colonies on bean seed coats (Braga*et al*., 2005). Men *et al.,* (2015) used bio-speckle to assess seed viability of *pisum sativum* seeds. Viable and non-viable *pisumsativum* seeds were illuminated by a helium-neon laser source of 7mW with wavelength of 632.8 nm. The speckle patterns were recorded by a digital colour charge-couple device camera and stored in the host computer for further analysis using Mat lab. The information of biological activities obtained from these speckle patterns can effectively utilized to distinguish viable and non-viable seeds.

Chlorophyll Fluorescence (CF) of Germinating Seeds

Development with CF of seeds is not just to measure the seed as a whole and obtain one figure, but to get values from over the entire surface of a seed: that is to say create a fluorescent image. Ideally this should again occur in an automated way and with large numbers of seeds at a time.

A set-up was created with high resolution cameras and special filtering producing the images in Fig. 12. It represents one pepper seed in a time sequence of 48 hours.

Fig.12. Chlorophyll Fluorescence of Germinating Pepper Seed showing Differential de novo Synthesis of Chlorophyll in a Seed Embryo. (Source: Plant Research International)

New technological developments make new applications possible. In dry and fully mature seeds the level of chlorophyll is relatively low, as we have seen; as soon as seeds start to imbibe, de novo synthesis of chlorophyll takes place. In this pepper seed the strongest signals come from the elongating hypocotyl and root base, followed by the cotyledons. This technology can, apart from being used for research purposes, also be developed into automated seed germination tests. CF provides ultra-clean images of seeds without background, which are ideal for image analysis (Burg, 2009).

Multispectral imaging for seed health testing

On wheat seed surface, presence of Fusarium spp., and black point disease (Alternaria spp.) infected parts could successfully be distinguished from uninfected parts with use of a multispectral imaging device. The multispectral images of 1,280 x 960 pixels were captured at 19 different spectral bands from visual (VIS) to near infra-red (NIR) wavelengths (375 nm—970 nm). After image capturing, the same seeds were incubated according to ISTA blotter test to confirm the presence of pathogen through microscopic analysis. Video meter software version 1.6, using normalized canonic discrimination analysis (nCDA) with all 19 bands, was used for data transformation in the multispectral images analysis. For visualization of data, a ROIs (Region of Interest) histogram was applied to illustrated differences in reflections intensity between identified groups (fig.12) (Vresak et al., 2016).

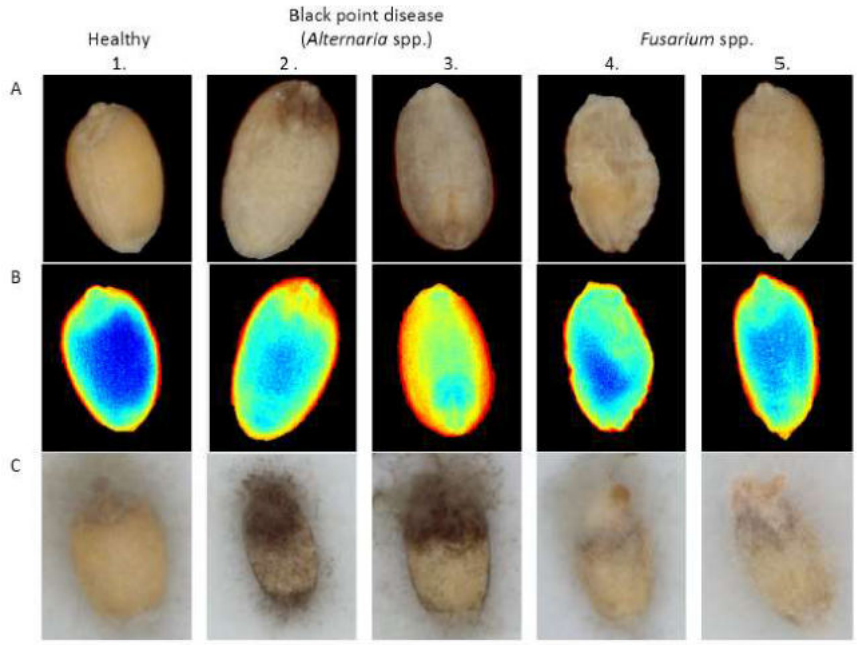


Fig 12. Image comparison of healthy seeds with infected seeds. Seeds on image were infected by black point disease (Alternaria sp.) and Fusarium sp. (A) RGB captured images. (B) nCDA-transformed images. (C) RGB images after seed incubation.

References

Braga R., Rabelo G.F., GranatoL.R.,Santos E.F., Machado J.C., Arizaga R., Rabal H.J., Trivi M. 2005. Detection of fungi in beans by the laser biospeckle technique. *Biosyst. Eng.* 91:465–469.

Burg, J.V.D. 2009. Raising seed quality: what is in the pipeline? Proceedings of the second world seed conference; Responding to the challenges of a changing world: The role of new plant varieties and high quality seed in agriculture (session 4). FAO Headquarters, Rome, September 8-10, 2009, pg: 177-186.

Dell'Aquila A. 2005. The use of image analysis to monitor the germination of seeds of broccoli (Brassica oleracea L.) and radish (Raphanus sativus L.), Ann. Appl. Biol. 146, 545–550.

Dell'Aquila, A. 2009.Digital imaging information technology applied to seed germination testing. A review. *Agron. Sustain. Dev*. 29:213–221.

Ducournau S., Feutry A., Plainchault P., Revollon P., Vigouroux B., Wagner M.H. 2004. An image acquisition system for automated monitoring of the germination rate of sunflower seeds, Comp. Electron. Agric. 44, 189–202.

DucournauS.,FeutryA.,PlainchaultP.,RevollonP., VigourouxB. 2005. Using computer vision to monitor germination time course of sunflower(*Helianthusannus*L.)seeds,*SeedSci.Technol*.33:329–340.

Ellis R.H., Roberts E.H. 1981.The quantification of ageing and survival in orthodox seeds, Seed Sci. Technol. 9, 373–409.

Fairchild M.D. 1998. Color appearance Models, Addison-Wesley, Reading, MA.

Garay, A. 2012. ISTA purity committee report 2012-progress in 2012 and future goal

Garay, A., Hanning, S. and Elias, S. 2010. Redesigning a purity testing system: development of an ergonomic, high-vision, continuous-flow seed inspection system. Seed testing international, ISTA News Bulletin.No139: 8-13.

Li L, Zhang Q, Huang D (2014) A review of imaging techniques for plant phenotyping. Sensors 14: 20078–20111

Men, S., Yan, L and Qian, H. 2015. Application of bio-speckle activity to assess seed viability. *Advance journal of food science and technology***8(3)**: 214-218.

Mo, C., Lim,J., Lee, K., Kang, S., Kim, M.S., Kim, G., Cho, B.K. 2013. Determination of germination quality of cucumber (*Cucumis sativus*) seed by LED-Induced Hyperspectral Reflectance Imaging. *J. of Biosystems Eng*. **38(4):**318-326.

Vrešak M, Halkjaer Olesen M, GislumR,Bavec F, RavnJørgensen J. 2016. The use ofimage-spectroscopy technology as a diagnosticmethod for seed health testing and varietyidentification. PLoS ONE 11(3): e0152011.

Wilkes T, Nixon G, Bushell C, Waltho A, Alroichdi A, Burns M (2016) Feasibility study for applying spectral imaging for wheat grain authenticity testing in pasta. Food Nutr Sci 7:355–361

*****

Advance Techniques to Detect Seed borne Pathogens and their Management

B.K. Sarma
Department of Mycology and Plant Pathology, Institute of Agricultural Sciences,
Banaras Hindu University, Varanasi

Seedborne pathogens present a serious threat to seedling establishment. Close association with seeds facilitates the long-term survival, introduction into new areas and widespread dissemination of pathogens. Under greenhouse conditions, the risks of significant economic losses due to diseases are great because factors including high populations of susceptible plants, high relative humidity, high temperatures and overhead irrigation, promote explosive plant disease development. Under these conditions, the most effective disease management strategy is exclusion which is accomplished by using seed detection assays to screen and eliminate infested seedlots before planting. The following will explore the current state of seed detection technology and include recent advances. A summary of the features of each assay is presented.

Conventional seed detection assays.

Testing seeds for plant pathogens can be a difficult task. Unlike infected vegetative plant tissues, infested seeds can be asymptomatic, making visual detection impossible. Additionally, pathogen populations on seeds may be low, and infested seeds may be nonuniformly distributed within a lot. Many detection assays exist for different seedborne 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. Seed assays have been developed based on different technologies including visual examination; selective media; seedling grow-out tests and serological techniques. While these tests have been used for many years, some of them have shortcomings that make them less than ideal. Brief descriptions of these assays including their advantages and disadvantages are discussed below.

Visual examination.

In some cases infected seeds display characteristic symptoms, including discoloration and shriveling. Examples of such seedborne diseases include purple seed stain (*Cercosporakikuchii*), and advanced stages of Phomopsis seed decay (*Phomopsis longicolla*) of soybean (*Glycine max*), and Cylindrocladium black rot (*Cylindrocladiumparasiticum*) of peanut (*Arachis hypogeae*). In these casesseedlot infestation can be reduced by using automatic devices that sort seeds based on visual of physical characteristics. These systems usually display low detection sensitivity. Additionally, seeds infested by fungi, bacteria and viruses may display no macroscopic symptoms, making visual or physical inspection of seeds useless as a detection assay.

Selective media.

A direct method of testing seeds is by allowing pathogens to grow from them onto appropriate artificial media. This can be done by directly plating surface-sterilized seed samples or seed-wash liquid onto artificial media, followed by incubation under adequate conditions. Once a pathogen is isolated it can be identified by its cultural or biochemical characteristics e.g. the production of a bluish-green fluorescent pigment on King's B medium in the case of fluorescent *Pseudomonas* spp. or the production of dark, muriform conidia in the case of *Alternaria* spp. Unfortunately, seeds may be contaminated by saprophytic microorganisms (nonpathogens) that grow as well as, or better than target organisms on nutrient-rich, artificial media. The excessive growth of saprophytic organisms including *Rhizopus* spp., *Penicillium* spp., and yeasts make it impossible to identify pathogens that may be present. The inability to identify the unique characteristics of the target pathogens in the presence of contaminating microorganisms lead to inaccurate assessments of seedlot infestation. To overcome this problem, selective artificial media are developed that use antibiotics, fungicides, selected carbon and nitrogen sources and other inhibitory compounds to retard the growth of nontarget microflora while allowing the pathogen to grow. Many selective and semiselective media have been developed for seedborne fungi and bacteria. Unfortunately, development of such media is time consuming and requires specific knowledge of the nutritional requirements and chemical tolerances of the target organism, relative to the nontarget seed microflora. Employing selective media also requires 2 to 4 d for pathogen growth and the test operator must be familiar with the range of cultural characteristics associated with the pathogen. Finally, while selective media can be applied for certain bacteria and fungi, it cannot be applied for nonculturable obligate parasites, e.g., viruses, nematodes and certain fungi and bacteria.

Seedling grow-out assay

The seedling grow-out assay is a direct measure of the seedlot's ability to transmit a disease. To conduct this assay, seedlot samples are planted under greenhouse conditions conducive to disease development and after germination, seedlings are observed for the development of symptoms. Seedling grow-out is one of the most applicable and widely used seed detection assays but for successful implementation, infected seedlings must display obvious and characteristic symptoms. Unfortunately, this is not always the case as some diseases have nondistinct symptoms, e.g., wilting, chlorosis, etc. Another drawback of the seedling grow-out assay is that large seed samples (10,000 to 50,000 seeds in the case of bacterial fruit blotch (*Acidovoraxavenae* subsp. *citrulli*) of watermelon (*Citrullus lanatus*) must be tested to statistically ensure that one infested seed can be detected. In addition to losses associated with the destructive testing of expensive seeds, assaying this quantity of seeds requires large areas of greenhouse space and adequate labor for assay set up and evaluation. The seedling grow-out assay is also time consuming, requiring up to weeks for seedling germination and symptom development. Finally, seed test evaluators must be familiar with the symptoms associated with each disease. This can be difficult since each disease has a range of possible symptoms that are influenced by environ-mental conditions. Hence, for the seed-ling grow-out assay, greenhouse conditions must be strictly regulated to ensure consistent results. In large greenhouses this can be a challenge and it can lead to erroneous test results. Also, because of the variations in seedling symptom expression it is

often necessary to isolate the pathogen from suspected seedlings for confirmation. These extra steps further prolong the time required to complete the seedling grow-out assay. Residual contamination and cross-contamination between spatially separated seedlots are also issues of concern under greenhouse conditions.

Blotter method

The collected seed samples need to analyze for the presence of major seed borne fungal the pathogens by blotter method following the International rules for Seed Testing. Seeds are tested for each variety maintaining four replications. Twenty-five seeds are placed on three layers of moist blotting paper (Whatman No.1) in each glass petridish. The petridishes are incubated at 25±1°C under 12/12 hrs light and darkness cycle for 7 days. Each seed is observed under strereomicroscope in order to record the presence of fungal colony and bacterial ooze 7 days after incubation based on growth habit. In doubtful cases temporary slides are prepared from the fungal colony observed under compound microscope. Appropriate keys are consulted for identification of the fungi and bacteria. The results are presented as percent incidence for individual pathogen. Germination of the seeds is also recorded. Each individual incubated seed is observed under stereomicroscope in order to record the incidence of seed borne fungi. Most of the associated pathogens were detected by observing their growth characters on the incubated seeds on blotter paper. For proper identification of fungi temporary slides are prepared from the fungal colony and observed under compound microscope and identified with the help of Keys. The fungi from the incubated seeds are also transferred to PDA when needed. The culture is incubated at 25±10°C for 3-7 days. Temporary semi permanent slides are prepared from the fungal colony and observed under compound microscope. The fungi are identified with the help of different books, manuals and publications. The results are presented as percent incidence for individual pathogen. acronyms written on the reverse.

Rolled paper towel method

Germinability of the seeds are determined in the laboratory at room temperature (30±2°C). 200 seeds are randomly taken from each variety and 40 seeds are placed between a pair of moist paper towels. There should be replications for each variety. The towels are rolled and the ends are closed by threads and covered by polyethylene paper to prevent drying. After 10 days of incubation period observations pertaining to (a) % germination, (b) Non germinated seed (hard seed and rotten seed), (c) Post-emergence death, (d) Shoot length (e) Root length (f) Vigor Index and (g) Incidence of different organism needs to be recorded. For determination of organisms some portion of the fungi growth on the infected seeds are taken with the needle and observed under compound microscope. For determination of seedlings vigour 10 seedlings (normal /abnormal) are randomly selected from each paper and their individual shoot and root length is measured. Length of shoot is measured from the base of the stem up to the growing point of the youngest leaf. Similarly, length of root is measured from the starting point of the root to the largest available lateral root apex. Vigour of the seedling is determined by the following formula:

Vigour Index = (mean of root length+ mean of shoot length) × percentage of seed germination.

Agar plate method

In the agar plate method, two hundred seeds are tested for each maintaining replications. Surface disinfected seeds (0.1% mercuric chloride) are plated on the PDA medium and the plated seeds are usually incubated for 5-7 days at 22-25°C under 12h altering cycles of light and darkness. At the end of the incubation period, fungi growing out from the seeds on the agar medium are examined and identified. Identification is done based on colony characters and morphology of sporulation structures under a compound microscope. In the agar plate method more than one type of fungal colonies are produced. In this case, identification is done on the most frequently occurring colony present in all the petridishes and then the second most frequent, the third most frequent and so on. Thereafter, the identification of the different colonies are done visually and then under a stereomicroscope and followed by an examination of the fruiting structures under a compound microscope. Once the identification is done, the colonies are assigned names and their acronyms written on the reverse [16].

Serology-based assays

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. Serology-based seed tests have several formats including the widely applied enzyme-linked immunosorbent assay (ELISA) and immunofluorescence microscopy. 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. Finally, with serology-based assays it is possible to detect nonviable pathogens which results in erroneous (false-positive) interpretation.

PCR-based assay

PCR-based assays exhibit very higher levels of sensitivity than any other conventional techniques. They require extraction of PCR-quality DNA from the target organisms in the background of saprophytic organisms and inhibitory seed-derived compounds when applied to seed tests. PCR consists *in vitro* enzymatic amplification of an initial quantity of target DNA from any living organisms including fungi, bacteria and viruses. However, due to its specificity, speed and sensitivity it has been used to diagnose many seed borne pathogens. However, high capital costs and technical expertise for establishing PCR capabilities is major obstacle in the PCR-based detection technique. The second major obstacle in successful implication of this method is false negatives (inhibition

of PCR reaction by various compounds contained in seeds) and false positives (amplification of DNA from non-viable cells) which restricts the accurate detection of the pathogen. Along with this its incapability in distinguishing between viable and non-viable cells is also one of the major constraints of this method.

Some Important Bacterial Seed Borne Pathogens

Crops	**Pathogens**
Wheat	*Pseudomonas syringae*pv. *syringae*, *Xanthomonas campestris* pv. *translucens*
Maize	*Pantoeastewartii*subsp. *stewartii*, *Clavibactermichiganensis* subsp. *nebraskensis*
Rice	*X. oryzae*pv. *oryzae, X. oryzae*pv. *oryzicola, Acidovoraxoryzae*
Bean	*P. syringae*pv. *phaseolicola, Curtobacteriumflaccumfaciens*pv. *flaccumfaciens, Xanthomonas campestris pv. phaseoli* and *X. fuscans var. fuscans*
Soybean	*P. syringae*pv. *glycinea*
Chickpea	*Rhodococcusfascians*
Cereals	*Rathayibacter*sp.
Alfalfa	*C. michiganensis*subsp. *insidiosus*
Tomato	*Pseudomonas syringae*pv. *tomato* (tomato), *P. syringae*pv.
Pepper	*syringae, Xanthomonas spp., Clavibactermichiganensis*subsp. *michiganensis*
Carrot	*Xanthomonas campestris* pv. *carotae*
Onion	*Pantoeaananatis, Burkholderiacepacia*
Crucifers	*Xanthomonas campestris* pv. *campestris*, *P. syringae*pv. *alisalensis*(broccoli) *Pseudomonas* spp. (crucifers)
Cucurbits	*P. syringae*pv. *lachrymans, Acidovoraxcitrulli*
Lettuce	*Xanthomonas campestris* pv. *vitians*

Some Important Fungal Seed Borne Pathogens

Crops	**Diseases**	**Pathogens**
Wheat	Loost smut	*Ustilago segetum* var. *tritci*
	Karnal smut	*Neovossiaindica*
	Flag smut	*Urocystisagropyri*
Chickpea	Ascochyta blight	*Ascochytarabiei*
	Wilt	*Fusarium oxysporum*f.sp. *ciceri*
Crucifers	Grey and black leaf	*Alterniabrassicae*
	Spot	*A. brassicicola*
Rice	Bunt	*Neovossia horrid*
	False Smut	*Ustilaginoideavirens*
	Stackburn	*Pyriculariaoryzae*
		Trichoconiellapadwickii

Cotton	Anthracnose	*Colletotrichum indicum*
	Wilt	*F. oxysporumf*.sp. *vasinfectum*
Maize	Black kernel rot	*Botryodiplodiatheobromae*
	Cob rot	*Fusarium moniliformae*
	Southern leaf blight	*Drechlera maydis*
Pearl millet	Downy mildew	*Sclerosporagraminicola*
	Smut	*Tolyposporiumpenicillsriae*
Sorghum	Anthracnose	*Colletotrichum graminicola*
	Kernel or grain smut	*Sphacelothecasorghi*
	Downy mildew	*Peronosclerosporasorghi*
Soybean	Anthracnose	*Colletotrichum dematium*
	Pod & stem blight	*Phomopsis sojae*
	Purple seed stain	*Cercosporakikuchii*
Cucumis spp.	Anthracnose	*Colletotrichum lagenarium*
Brinjal	Fruit rot	*Phomopsis vexans*
Onion	Damping off	*Botrytis allii*
	Downy mildew	*Peronospora destructor*
	Purple blotch	*Alterniaporri*
	Stemphylium Blight	*Stemphylium vesicarium*
Pepper chilies	Anthracnose Or ripe fruit rot	*Colletrotrichumcapsici*
Tomato	Buck eye rot	*Phytophthora parasitica*
	Damping off	*Phythiumaphanidermatum*
	Early Blight	*Alterniasolani*
	Late blight or Fruit rot	*Phytopthorainfestans*

*****

Physical purity and ODV testing of seed samples through Image Analysis System

S. K. Chakrabarty, Principal Scientist,
Division of Seed Science and Technology, IARI, Pusa, New Delhi

Physical purity analysis tells us the of pure seed component in the seed lot as well as the proportion of other crop seed, weed seed and inert matter by weight in percentage for which Seed Standards have been prescribed. Thus it helps in:

i) Improving the plant stand (by increasing the pure seed component)
ii) Raising a pure crop (by eliminating other crop seed and weed seeds)
iii) Raising a disease free-crop (by eliminating other crop seed and weed seeds)
iv) In the use of seed drill (by selecting uniform particles)

There is a need for physical purity analysis for:

a) Seed Certification of Seed law Enforcement Agencies to judge that the seed lot conforms to the prescribed standards
b) Seed processing plants for using right kind of processing equipment
c) Physical purity analysis is a prerequisite for germination test because 'pure seed' component is used for germination testing

Objective

The primary objective of physical purity analysis is to determine i) the percentage composition by weight of the sample being tested and by inference the composition of lot and ii) the identity of the various species of seed and inert particles constituting the sample. The definitions of various physical purity components in the ISTA rules are as follows:

Pure Seed

1. The pure seed refers to the species stated by the sender, or found to predominate in the test, and shall include all botanical varieties and cultivars of that species (even if immature, undersized, shrivelled, diseased and germinated, provided they can be definitely identified as of that species) unless transformed into visible sclerotia, smut balls or nematode galls. Pure seed includes: a) Intact seed units (commonly found as dispersal units i.e. achenes and similar fruits, schizocarp florets etc.) as defined for each genus or species; b) pieces of seed units larger than one half their original size. In the case of pieces of seeds, any piece which is larger than one-half the original size shall be considered pure seed except that seeds of the Fabaceae and Brassicaceae with their seed coats entirely removed shall be regarded as inert matter.

From the above main principles certain exceptions made for particular genera or species are as follows:

i) Seed units of families namely, *Leguminosae, Cruciferae, Cupressaceae, Pinaceae and Taxodiaceae* with the seed coat entirely removed shall be regarded as inert matter. Separated cotyledons of Leguminosae are regarded as inert matter, irrespective of

whether or not the radical-plumule axis and/or more than half of the testa may be attached.

ii) In certain genera of family like Poaceae (Gramineae)

a) A minimum size of caryopsis is required i.e. in *Lolium, Festuca and Agropyronrepens* a floret with a caryopsis one third or more of the length of palea measured from the base of rachilla is regarded as pure seed, but a caryopsis less than 1/3 the length of the palea is regarded as inert matter.

b) The presence of caryopsis is spikelet and floret is not always obligatory.

iii) In the case of the florets and caryopses of Poaceae, pure seed shall consist of:

iii) Broken florets or free caryopses, provided they are larger than one-half the original size,

iv) Entire florets and one-seeded spikelets with an obvious caryopsis containing endosperm, as determined by the use of slight pressure or by examination over light,

v) In the case of the Festuca spp., Agropyroncristatum, Agropyrondesertorum or Agropyron fragile attached sterile florets which do not extend to or beyond the tip of the fertile floret shall be left attached and considered part of the pure seed. The length of an awn shall be disregarded when determining the length of a sterile floret,

vi) The separation of pure seed and inert matter is done by uniform blowing procedure. This method is obligatory for Poa pratensis and Dactylisglomerata and it is recommended for Chloris gayana as an alternative to hand method.

vii) Multiple seed units (MSU) are left intact in the pure seed fraction e.g. Dactylia and Festuca.

viii) Attached sterile florets are not removed, but left attached and included in the pure seed fraction e.g. Arrhenatherum, Avena, Chloris, Dactylis, Festuca, Holeus, Poa and sorghum. This applies to the attached sterile florets of *Lolium* which do not extend to the tip of the fertile floret, excluding the awn.

iv) Where the Uniform Blowing Method is used, all material of the kind of seed under analysis which remains in the heavy portion after blowing according to the instructions for that kind of seed, not including:

- Broken florets or free caryopses which are one-half or less than one-half of the original size,
- Other crop seeds,
- Weed seeds,
- Heavy inert matter,
- In the case of *Dactylisglomerata*, one-fifth the weight of multiple florets (see *Uniform Blowing Method*).

v) Florets with fungus bodies, such as ergot (*Clavicepspurpurea*), entirely enclosed within lemma and palea; and

vi) Four-fifths the weight of multiple florets remaining in the heavy portion in the case of *Dactylisglomerata*.

Other crop Seed

Other crop seed includes seed units of any plant species other than of pure seed grown as crop. Multiple structures, capsules, pods are opened and the seeds are taken out and the non-seed material is placed in the inert matter.

Weed Seed

Seeds, bulblets or tuber of plants recognized by laws, official regulations or by general usage are considered as weed seeds.

Inert Matter

Inert matter includes seed units and all other matter and structures not defined as pure seed excluding other crop seed and weed seeds.

General Principles

As per ISTA rules, the working sample is separated into three components i.e., pure seeds, other seeds and inert matter. The percentage of each part is determined by weight. All species of seed and each kind of inert matter present are identified as far as possible and if required for reporting, its percentage by weight shall be determined.

1. Equipments

Aids such as transmitted light, sieves and blowers may used is separating the component parts of the working sample. The blower is to be used for the uniform blowing for species of family Poaceae (Gramineae).

Other equipments required are:

1. Dividers - a) Soil type - works on gravitational force
b) Boerner - works on gravitational force
c) Gamete - works on centrifugal force and electrically operated.

2. Balance: Electric or electronic balances are better due to their accuracy & quickness.
3. Blowers
4. Diaphanoscope using reflected light are used to separate inert matter such as empty florets of grasses.
5. Sieves
6. Sample pans, dishes, forceps, spatula and hand lens.
7. Seed herbarium of crop and weed seeds.

Procedure

1. Obtaining Working Sample

Since the size of the working sample is minute as compared as compared with the size of the seed lot to which it represent, it is therefore, very essential that the working sample should be obtained in accordance with procedures. The working sample shall be either a weight estimated to contain at least 2,500 se3ed units or not less than weight indicated eg. 40 g for *Oryza sativa*.

Boerner or soil type seed divider should be used to homogenize the submitted sample before reducing it to the size of working sample. The following guidelines need to be followed:

a) Check the cleanliness of the divider and the container.

b) Pour the entire contents of the submitted sample into the hopper of the divider.

c) Allow the contents of the submitted sample to pass through the main body of the divider. In case of "Soil type" seed divider this can be accomplished by tilting the hopper over the body of the divider while in case of "Boerner" divider, by operating the gate-value situated at the base of the hopper.

d) Recombine the contents of both sample receiving pans and again pass it through the divider.

e) Repeat this process twice in order to homogenize the submitted sample. Divide the submitted sample.

f) Divide the submitted sample.

g) Set aside the contents of one container.

h) Divide the contents of the other container subsequently till the weight of working sample is obtained.

2. Separation

a) Clean the work board, sample pans and purity dishes before starting the separation.

b) Examine the working sample to determine the use of particular aid such as blower or sieves for making separation.

c) After preliminary separation with the help of sieves of blower, place and spread the retained or heavier portion (A) on the purity work board.

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

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

f) Seed enclosed in fruits other than those indicated in pure seed should be separated and detached empty fruit/appendages classified as inert matter.

g) Collect the pure seed in the sample pan.

h) Put the lighter portion (B) of the working sample on the work board and examine under magnification for further separating into the requisite classes (other crops seed, weed seed and inert matter).

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

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

Sl. No.	Weight of working Sample (g)	No. of decimal place required	Example

1.	Less than 1	4	0.9025
2.	1 to 9.990	3	9.025
3.	10 to 99.99	2	90.25
4.	10 to 999.9	1	902.5
5.	1000 or more	0	1025

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

3. Reporting Results

If the weight of the component fractions from working sample is more than 5% the initial weight of the working sample, a retest must be done. The result of the retest is then reported.

i) The percentage weight of the components shall be given to one decimal place and must total to 100.0%. If the sum of the total component percentages does not equal 100.0% then add or subtract 0.1% from the largest value (normally the pure seed fraction).

ii) Results of less than 0.5% shall be reported as trace or 'TR'. Generally, percentages are rounded to single decimal place (0.05% to 0.09% shall be reported as 0.1%).

iii) If the percentage for a component is 'nil', this must be shown as '0.0' in appropriate space.

iv) For reporting under ISTA Certificates, procedure given in latest ISTA Rules for Seed Testing (Clause 3.7) shall be strictly followed.

4. Tolerances

I. The following tables are considered for comparing purity test results;

II. **Table-5.1:** Tolerances for comparing purity test results on duplicate samples from the same submitted sample in the same laboratory

III. **Table-5.2 & 5.3:** Tolerances for comparing purity test results on two different submitted samples from the same seed lot when a second test is made in the same laboratory.

IV. Table-5.1: Tolerance for purity tests on the same submitted samples in the same laboratory (two-way test at 5% significance level)

This Table gives tolerances for comparing purity results on duplicate samples from the same submitted sample analyzed in the same laboratory. It can be used for any component of a purity test. The Table is used by entering it at the average of the two test results (Column 1 or 2). The appropriate tolerance is found in one of the Columns 3 to 6, determines as to whether the seeds are chaffy or non chaffy and half or whole working samples have been analyzed.

The tolerances in column 5 and 6 are extracted from Miles (1963).

Average of the two test results		**Tolerances for differences between**			
		Half working sample		**Whole working sample**	
		Non chaffy seeds	**Chaffy seeds**	**Non chaffy seeds**	**Chaffy seeds**
(1)	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**
99.95-100.00	0.00-0.04	0.20	0.23	0.1	0.2
99.90-99.94	0.05-0.09	0.33	0.34	0.2	0.2

99.85-99.89	0.10-1.14	0.40	0.42	0.3	0.3
99.80-99.84	0.15-0.19	0.47	0.49	0.3	0.4
99.75-99.79	0.20-0.24	0.51	0.55	0.4	0.4
99.70-99.74	0.25-0.29	0.55	0.61	0.4	0.4
99.65-99.69	0.30-0.34	0.61	0.65	0.4	0.5
99.60-99.64	0.35-0.39	0.65	0.68	0.5	0.5
99.55-99.59	0.40-0.44	0.68	0.72	0.5	0.5
99.50-99.54	0.45-0.49	0.72	0.76	0.5	0.5
99.40-99.49	0.50-0.59	0.76	0.82	0.5	0.6
99.30-99.39	0.60-0.69	0.83	0.89	0.6	0.6
99.20-99.29	0.70-0.79	0.89	0.95	0.6	0.7
99.10-99.19	0.80-0.89	0.95	1.00	0.7	0.7
99.00-99.09	0.90-0.99	1.00	1.06	0.7	0.8
98.75−98.99	1.00-1.24	1.07	1.15	0.8	0.8
98.50−98.74	1.25-1.49	1.19	1.26	0.8	0.9
98.25−98.49	1.50-1.74	1.29	1.37	0.9	1.0
98.00−98.24	1.75-1.99	1.37	1.47	1.0	1.0
97.75−97.99	2.00-2.24	1.44	1.54	1.0	1.1
97.50−97.74	2.25-2.49	1.53	1.63	1.1	1.2
97.25−97.49	2.50-2.74	1.60	1.70	1.1	1.2
97.00-97.24	2.75-2.99	1.67	1.78	1.2	1.3
96.50−96.99	3.00-3.49	1.77	1.88	1.3	1.3
96.00−96.49	3.50-3.99	1.88	1.99	1.3	1.4
95.50−95.99	4.00-4.49	1.99	2.12	1.4	1.5
95.00−95.49	4.50-4.99	2.09	2.22	1.5	1.6
94.00−94.99	5.00-5.99	2.25	2.38	1.6	1.7
93.00−93.99	6.00-6.99	2.43	2.56	1.7	1.8
92.00−92.99	7.00-7.99	2.59	2.73	1.8	1.9
91.00−91.99	8.00-8.99	2.74	2.90	1.9	2.1
90.00−90.99	9.00-9.99	2.88	3.04	2.0	2.2
88.00−89.99	10.00-11.99	3.08	3.25	2.2	2.3
86.00−87.99	12.00-13.99	3.31	3.49	2.3	2.5
84.00−85.99	14.00-15.99	3.52	3.71	2.5	2.6
82.00−83.99	16.00-17.99	3.69	3.90	2.6	2.8
80.00−81.99	18.00-19.99	3.86	1.07	2.7	2.9
78.00−79.99	20.00-21.99	4.00	4.23	2.8	3.0
76.00−77.99	22.00-23.99	4.14	4.37	2.9	3.1
74.00−75.99	24.00-25.99	4.26	4.50	3.0	3.2
72.00−73.99	26.00-27.99	4.37	4.61	3.1	3.3
70.00−71.99	28.00-29.99	4.47	4.71	3.2	3.3
65.00−69.99	30.00-34.99	4.61	4.86	3.3	3.4
60.00−64.99	35.00-39.99	4.77	5.02	3.4	3.6

50.00−59.99	40.00-49.99	4.89	5.16	3.5	3.7

V. Table-5.2: Tolerance for purity tests on two different submitted samples from the same lot when a second test is made in the same or different laboratory (one-way test at 1% significance level).

This Table gives tolerances for comparing purity results on duplicate samples from the same submitted sample analyzed in the same laboratory. It can be used for any component of a purity test. The Table is used by entering it at the average of the two test results (Column 1or2). The appropriate tolerance is found in one of the Columns 3 to 6, determines as to whether the seeds are chaffy or non chaffy and half or whole working samples have been analyzed. The tolerances in column 3 and 4 are extracted from Miles (1963).

Average of the two test results		**Tolerances**	
50-100%	**Less than 50%**	**Non chaffy seeds**	**Chaffy seeds**
(1)	**(2)**	**(3)**	**(4)**
99.95-100.00	0.00-0.04	0.2	0.2
99.90-99.94	0.05-0.09	0.3	0.3
99.85-99.89	0.10-0.14	0.3	0.4
99.80-99.84	0.15-0.19	0.4	0.5
99.75-99.79	0.20-0.24	0.4	0.5
99.70-99.74	0.25-0.29	0.5	0.6
99.65-99.69	0.30-0.34	0.5	0.6
99.60-99.64	0.35-0.39	0.6	0.7
99.55-99.59	0.40-0.44	0.6	0.7
99.50-99.54	0.45-0-49	0.7	0.7
99.40−99.49	0.50-0.59	0.7	0.8
99.30−99.39	0.60-0.69	0.8	0.9
99.20−99.29	0.70-0.79	0.8	0.9
99.10-99.19	0.80-0.89	0.9	1.0
99.00−99.09	0.90-0.99	0.9	1.0
98.75−98.99	1.00-1.24	1.0	1.1
98.50−98.74	1.25-1.49	1.1	1.2
98.25−98.49	1.50-1.74	1.2	1.3
98.00−98.24	1.75-1.99	1.2	1.4
97.75−97.99	2.00-2.24	1.3	1.5
97.50−97.74	2.25-2.49	1.3	1.6
97.25−97.49	2.50-2.49	1.4	1.6
97.00-97.24	2.75-2.99	1.5	1.7
96.00−96.49	3.50-3.99	1.6	1.9
95.50−95.99	4.00-4.49	1.7	2.0
95.00−95.49	4.50-4.99	1.8	2.2
94.00−94.99	5.00-5.99	2.0	2.3
93.00−93.99	6.00-6.99	2.1	2.5

92.00−92.99	7.00-7.99	2.2	2.6
91.00−91.99	8.00-8.99	2.4	2.8
90.00−90.99	9.00-9.99	2.5	2.9
88.00−89.99	10.00-11.99	2.7	3.1
86.00−87.99	12.00-13.99	2.9	3.4
84.00−85.99	14.00-15.99	3.0	3.6
82.00−83.99	16.00-17.99	3.2	3.7
80.00−81.99	18.00-19.99	3.3	3.9
78.00−79.99	20.00-21.99	3.5	4.1
76.00−77.99	22.00-22.99	3.6	4.2
74.00−75.99	24.00-25.99	3.7	4.3
72.00−73.99	26.00-27.99	3.8	4.4
70.00−71.99	28.00-29.99	3.8	4.5
65.00−69.99	30.00-34.99	4.0	4.7
60.00−64.99	35.00-39.99	4.1	4.8
50.00−59.99	40.00-49.99	4.2	5.0

VI. **Table-5.3: Tolerance for purity tests on two different submitted samples from the same lot when a second test is made in the same or different laboratory (two-way test at 1% significance level).**

VII. This Table gives the tolerance for purity results made on the different submitted samples each drawn from the same lot and analyzed in the same or a different laboratory. It can be used for any component of a purity test to decide whether two estimates are compatible. The Table is used by entering it at the average of the two test results (Column 1 or 2). The appropriate tolerance is found in column 3 or 4, determined by whether the seeds or chaffy on non-chaffy. The tolerance in columns 3 and 4 are extracted from columns D and G, respectively of Table P7 Miles (1963).

VIII.

Average of the two test results		**Tolerances**	
50-100%	**Less than 50%**	**Non chaffy seeds**	**Chaffy seeds**
(1)	**(2)**	**(3)**	**(4)**
99.95-100.00	0.00-0.04	0.2	0.2
99.90-99.94	0.05−0.09	0.3	0.4
99.85-99.89	0.10−0.14	0.4	0.5
99.80-99.84	0.15−0.19	0.4	0.5
99.75-99.79	0.20-0.24	0.5	0.6
99.70-99.74	0.25−0.29	0.5	0.6
99.65-99.69	0.30−0.34	0.6	0.7
99.60-99.64	0.35−0.39	0.6	0.7
99.55-99.59	0.40−0.44	0.6	0.8
99.50-99.54	0.49 −0.49	0.7	0.8
99.40-99.49	0.50−0.59	0.7	0.9
99.30-99.39	0.60−0.69	0.8	1.0

99.20-99.29	0.70−0.79	0.8	1.0
99.10-99.19	0.80−0.89	0.9	1.1
99.00-99.09	0.90−0.99	0.9	1.1
98.75-98.99	1.00−1.24	1.0	1.2
98.50-98.74	1.25−1.49	1.1	1.3
98.25-98.45	1.50−1.74	1.2	1.5
98.00-98.24	1.75−1.99	1.3	1.6
97.75-97.99	2.00−2.24	1.4	1.7
97.50-97.74	2.25−2.49	1.5	1.7
97.25-97.49	2.50−2.74	1.5	1.8
97.00-97.24	2.75-2.99	1.6	1.9
96.50-96.99	3.00−3.49	1.7	2.0
96.00-96.49	3.50−3.99	1.8	2.1
95.50-95.99	4.00−4.49	1.9	2.3
95.00-95.49	4.50−4.99	2.0	2.4
94.00-94.99	5.00−5.99	2.1	2.5
93.00-93.99	6.00−6.99	2.3	2.7
92.00-92.99	7.00−7.99	2.5	2.9
91.00-91.99	8.00−8.99	2.6	3.1
90.00-90.99	9.00−9.99	2.8	3.2
88.00-89.99	10.00−11.99	2.9	3.5
86.00-87.99	12.00−13.99	3.2	3.7
84.00-85.99	14.00−15.99	3.4	3.9
82.00-83.99	16.00−17.99	3.5	4.1
80.00-81.99	18.00−19.99	3.7	4.3
78.00-79.99	20.00−21.99	3.8	4.5
76.00-77.99	22.00−22.99	3.9	4.6
74.00-75.99	24.00−25.99	4.1	4.8
72.00-73.99	26.00−27.99	4.2	4.9
70.00-71.99	28.00−29.99	4.3	5.0
65.00-69.99	30.00−34.99	4.4	5.2
60.00-64.99	35.00−39.99	4.5	5.3
50.00-59.99	40.00−49.99	4.7	5.5

Structures classified as Pure Seed in Purity Analysis for some Important Crop Plants
Poaceae

Oryza sativa(Paddy)

1. Spikelet, with glumes, lemma and palea enclosing a caryopsis, excluding entire awn when the length of awn is longer that the length of floret.
2. Floret, with or without sterile lemmas, with lemma and palea enclosing a cayopsis, excluding e4ntire awn when the length of awn is longer than the length of floret.
3. Caryopsis
4. Piece of caryopsis larger than one-half the original size.

Hordeum (Barley)

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

Avena(Oat)

1. Spikelet with lemma and palea enclosing a caryopsis, with or without awn plus attached sterile floret.
2. Floret with lemma and palea enclosing a caryopsis, with or without awn.
3. Caryopsis
4. Place of caryopsis larger than one-half the original size.

Triticum, Zea, Secale (Wheat, Maize, Triticale)

1. Caryopsis
2. Piece of caryopsis larger than one-half the original size.

Panicum (Sawa)

1. Spikelet with glumes, lemma and palea enclosing a caryopsis, plus sterile lemma.
2. Florets with lemma and palea enclosing a caryopsis.
3. Piece of caryopsis larger than one-half the original size.

Pennisetum (pearlmillet)

1. Fascicle of 1-5 spilelets (spilelets (spikelets with glumes, lemma and palea enclosing a caryopsis, plus attached sterile lemma) with involucre of bristles.
2. Floret with lemma and palea enclosing a cryopsis
3. Caryopsis
4. Piece of caryopsis larger than one-half the original size

Sorghum (Jowar)

1. Spikelet with glumes enclosing a cryopsis with or without hyaline palea or Floret, with lemma and palea, with or without awn.
2. Caryopsis
3. Piece of caryopsis larger than one-half the original size

Leguminaceae(Gram, Pea, Mung, Urd, Bean, Cluster bean, Soyabean, Lupins, (*Crotlaria* (Sunhemp), *Medicago, Arachis, Trifolium*)

1. Piece of seed larger than one-half the original size, with testa.
2. Seeds and pieces of seed without testa is regarded as inert matter.
3. Seperated cotyledons are regarded as inert matter irrespective of whether or not the redicle plumule axis/or more than half of the testa may be attached.

Cruciferae (Radish, Mustard, Cabbage and Cauliflower)

1. As given in 'Leguminaceae'

Solanaceae (Chillies, Brinjal, Tomato, Tobacco)

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

Linaceae (Linum)

1. As given in 'Solanancease'

Liliaceae (Onion, Garlic)

1. As above

Amaranthaceae *(Amaranthus)*

Cucurbitaceae (Watermelon, Longmelon, Muskmelon, Cucumber, Pumpkin, Squash, Bottle guard)

1. As above

Pediliaceae (Sesamum)

1. As above

Cannabaceae (Cannabis)

1. As above

Tiliaceae (Corchorus)

1. As above

Convolvaaceae(Ipomea)

1. As above

Umbellifer*ae* (Carum, Coriander, Cumin, Carrot, Fennel)

Schizocarp is a dry fruit which seperates into two or more units (mericarps) at maturity.

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

Malvaceae(cotton)

1. Seed with or without testa (testa with or without fuzz)
2. Piece of seed larger than one-half the original size with or without testa

Compositeae(Sunflower, Lettuce, Chicory)

1. Achene, with or without pappus, unless it is obvious that no seed is present.
2. Piece of achene larger than one-half the original size, unless it is obvious that no seed is present.
3. Seed with the pericarp/testa partially or entirely removed.
4. Piece of seed larger than one half the original size, with the pericarp/testa partially or entirely removed

Euphorbiaceae (*Ricinus*)

1. Seed with or without testa, with or without caruncle.
2. Piece of seed larger than one half the original size with or without testa

Chenopodiaceae(Spinach beet, Sugar beet, Red beet)

1. Cluster, or piece of cluster, including attached stalk unless it is obvious that no seed is present.
2. Seed, with pericarp, testa partially or entirely removed.
3. Piece of seed larger than ½ the original size with the pericarp/testa partially or entirely removed.
4. Seed with pieces of stalk protruding more than the width of cluster are reported according to Rule 3.7. [When a particular kind of inert matter, species of other seed, multiple seed unit (MSU) or seeds with appendages attached is found to the extent of the 1% or more, the percentage of such material must be shown on the analysis certificate].
5. In certain genera seeds/fruits may have various appandages (awns/stalks etc.) attached. Such appendages shall be left attached to the seeds, but the content of seeds with appendages longer than the greatest dimensions must be reported on the certificate.

Determination of ODV

Objectives

I. Determine the extent of seeds of other distinguishable varieties
II. Determination of other distinguishable characters shall be done only on the basis of readily apparent differences in the stable morphological characteristics of seeds.
III. The morphological characters are determined with the aid of a suitable magnifier
IV. The colour characteristics are determined under full day light of the limited spectrum
V. The availability of authentic sample o breeder seed (for comparison) is must for this determination

Morphological Characters

- Size and shape of the grain
- Base of lemma
- Wrinkling of lemma and palea
- Deviation of lateral dorsal nerves
- Rachilla hairs

- Shape and hairiness of lodicules
- Seed coat bloom
- Hilum colour (dicot)
- Seed colour

Methodology

- Place the seeds on Purity working board
- Examine each seed with the aid of magnifying lens
- Separate the seeds differ in morphological characteristics
- Count the number
- Express ODV by Number per Kg. for all cropsexcept rice
- Forrice ODV is expressed by Weight /Wei.ht as percentage

ODV limits

Crop	Foundation	Certified
Paddy	0.05%	0.2%
Sorghum	10	20
Maize	5	10
Pigeonpea	10	20
Blackgram	10	20
Greengram	10	20
Cowpea	5	10
Chickpea	5	10
Pea	5	10
Sesame	10	20
Castor	5	10
Soybean	10	20

*****

Testing of Coated and Pelleted Seeds

P. M. Singh
Principal Scientist, Indian Institute of Vegetable Research, Varanasi

Coated or pelleted seeds are to be dealt with appropriately in order to make positive identification of all individual seeds and inert matter which is impracticable without destroying the structures presented for testing. A wide range of materials may be used to coat seeds as individuals in discrete units as in pellets or spaced in strips or sheets. Where reference is made to seed pellets the rules also apply to encrusted seed and seed granules to seed tapes or to seed mats. For seeds thus coated, modified techniques are recommended to test the seeds, based on specific instructions.

Definitions

Seed pellets: More or less spherical units developed for precision sowing, usually incorporating a single seed with the size and shape of the seed no longer readily evident. The pellet, in addition to the pelleting material, may contain pesticides, dyes or other additives.

Encrusted seed: Units more or less retaining the shape of the seed with the size and weight changed to a greater or lesser extent. The encrusting material may contain pesticides, fungicides, dyes or other additives.

Seed granules: Units more or less cylindrical, including types with more than one seed joined together. The granule, in addition to the granulating material, may contain pesticides, dyes or other additives.

Seed tapes: Narrow bands of material, such as paper or other degradable material, with seeds spaced randomly, in groups or in a single row.

Seed mats: Broad sheets of material, such as paper or other degradable material, with seeds placed in rows, groups or at random throughout the sheets.

Treated seed: Seed to which only pesticides, dyes or other additives have been applied which have not resulted in a significant change in size, shape or addition to the weight of the original seed and which can still be tested according to the methods prescribed in other chapters.

Sampling Size of lot

The lot should be reasonably homogeneous; the maximum weight of lot may be as great as the maximum weight of lot which sampling procedures are prescribed. Subject to the tolerance of 5% and subjected to the seed number limitation.

Seed taps or seed mats may contain a maximum number of seeds of 1,00,000,000 (10,000) except that the weight of lot, including the coating material may not exceed 42,000 kg (40,000 kg plus 5%) when lot size is expressed in units the total weight of the lot must be given on the Certificate.

Sampling intensity

Sampling the lot of seed pellets should be done according to the intensity appropriate to the particular lot. Sampling the lot of seed tapes should be done by taking

packets or (from reels) pieces of tape at random analogously, following the prescriptions of 2.6.2 and 2.6.2. A., provided that packets or reels containing up to 2,000,000 (20 units of 100,000) seeds, may be combined as a basic unit and therefore are to be considered as one container.

Size of submitted sample:-

Submitted samples shall contain not less than the number of pellets or seeds indicated in column 2 of table given below. If a smaller sample is used the following statement must be inserted on the Certificate: "The Sample submitted contained only…….. pellets (seeds) and is / not in accordance with the International Rules for seed Testing".

Table 1: Part I: Sample size of pelleted seeds in number of pellets

Determinations	Submitted samples not less than	Working samples not less than
Purity analysis (including verification of species)	7500	2500
Weight determination	7500	Pure pellet fraction
Germination	7500	400
Determination of other seeds	10000	7500
Determination of other seeds (encrusted seeds and seed granules)	25000	25000
Size grading	10000	2000

Table 1 : Part II : Sample size of seed tapes

Determinations	Submitted samples not less than	Working samples not less than
Verification of species	2500 seeds	100 seeds
Germination	2500 seeds	400 seeds
Purity analysis (if required)	2500 seeds	2500 seeds
Determination of other seeds	10000 seeds	7500 seeds

Drawing and disposed of submitted sample: As submitted samples of coated seeds normally contain fewer seeds than corresponding samples of uncoated seeds, special care is necessary in drawing the sample to ensure that it is representative of the lot. Precautions are necessary to avoid damage to or change in the pellets or seed tape during drawing, handling and transport, and samples must be submitted in suitable containers.

Size of working sample: Working samples shall contain not less than the number of pellets or seeds indicated in column 3 of Table 1 Part I and Part II. if a smaller sample is used the actual number of pellets or seeds in the sample shall be reported on the Analysis Certificate.

Obtaining the working sample: For pelleted seeds, use one of the dividers described viz., conical divider, soil divider or centrifugal divider of fall must never exceed 250 mm. For seed tapes take pieces of tape at random, to provide sufficient seeds for the test.

Purity analysis

A purity analysis in the strict sense (i.e. of the seeds inside the pellets and tapes) is not obligatory though. If requested by the sender, a purity analysis on depelleted seeds or

seed removed from tape may be carried out to separate the sample into different parts, to determine the percentage of each part by weight. Separations for pelleted seed are defined but for taped seed no separation is made.

Pure pellets: Pure pellets shall include:

- Entire pellets regardless of whether or not they contain seed,
- Broken and damaged pellets in which more than half the surface of the seed is covered by pelleting material, except when it is obvious that either the seed is not of the species stated by the sender or there is no seed present.

Unpelleted seed: Unpelleted seed shall include:

- Free seed shall include :
- Broken pellets containing a seed that is recognizably not of the species stated by the sender.
- Broken pellets containing seed recognizable as being of the species stated by the sender but not included in the pure pellets fraction.

Inert matter: Inert matter shall include:

- Loose pelleting material
- Broken pellets in which it is obvious that there is no seed
- Any other material defined as inert matter in Rule.

Verification of species in pelleted Seeds:

In order to check that the seed in the pellets is largely of the species stated by the sender, it is obligatory to remove the pelleting material from 100 pellets taken from the pure pellet fraction of the purity test and determine the species of each seed. The pelleting material may be washed off or removed in the dry state. Similarly 100 seeds must be removed from tapes and the identity of each seed determined.

Procedure:

The pelleting material may be washed off or removed in the dry state. For taped seed, depending on the material the tape is made of, strip off or dissolve away the tape so that 100 seeds can be examined. When the seeds in the tape are also pelleted remove the pelleting material as indicated above.

Working sample

For pellets the purity analysis shall be made on a working sample taken from the submitted sample. The size of the working sample shall be that indicated in column 3 of Table 11A, Part I. The analysis may be made on one working sample of this number of pellets or on two sub-samples of at least half this number each independently drawn. The working sample (or each sub-sample) shall be weighed in grams to the minimum number of decimal places necessary to calculate the percentage of its component parts to one decimal place.

1. Procedures for purity tests on depelleted seeds and seeds removed from tapes

When a purity test on depelleted seeds is to be undertaken at the request of the sender the working sample of not less than 2500 pellets is depelleted by shaking in fine mesh sieves in water. A sieve of 1.00 mm mesh above a sieve of 0.5 mm is recommended. The pelleting material is dispersed in the water and the remaining seed material is dried overnight on filter paper and then in an air oven at the temperature

indicated for the species under test. After drying, the material must be subjected to a purity analysis. The component parts (Pure seed, other seeds and inert matter) shall be reported as percentages of their total weight, ignoring the pelleting material. The percentage of pelleting material shall be reported separately only on request.

When a purity test on seeds removed from tapes is requested, the tape material of the working sample with paper tapes is cautiously separated and stripped off. Water soluble tape material is moistened until the seeds come free. When pelleted seeds are found in the tapes follow the procedure in the paragraph above. The moistened seeds must be dried and the free seed material must be subjected to a purity test as above. The component parts (Pure seed, other seeds and inert matter) shall be reported as percentages of their total weight ignoring the tape material.

The results of these tests are to be reported under 'Other Determinations' and endorsed 'weight of …….. Material excluded".

Separation

The working sample of pellets (or sub-sample) after weighing is separated into its components.

Calculation and expression of results

The percentage by weight of each of the component parts shall be calculated one decimal place. Percentages must be based on the sum of the weights of the components, not on the original weight of the working sample, but the sum of the weights of the components must be compared of the working sample, but the sum of the weights of the components must be compared to the original weight as a check against loss of material or other error.

Reporting results

The result of a purity analysis shall be given to one decimal place and the percentage of all components must total 100. Components of less than 0.05% shall be reported as trace.

The name and number of seeds of each species found in the examination of the 100 seeds removed from pellets or tapes shall be reported on the International Seed Testing Association International Seed Analysis Certificate under 'Other Determinations'.

The percentage of pure pellets, unpelleted seed and inert matter must be reported in the spaces provided on the International Seed Analysis Certificate for the components of the purity test on unpelleted seed.

2. Determination of other seeds:

This determination to estimate the number of seeds of other species is carried out only at the request of the sender.

In determining the number of other seeds, the definition prescribed in Rule 3.2 shall be observed. Other seeds refer to species other than that of the pure seed as defined in Rule 3.2.1.

General principles

The determination is made by a count of seeds of the species (or groups of species) designated by the sender, and the result is expressed as a number of seeds found in the weight and approximate numbers of pellets examined or for tapes in the length of tape (or area of mat) examined.

Procedure

Working sample

The working sample shall be not less than that prescribed in column 3 of Table 11 A, part 1 and Part 2. The working sample of pellets may be divided into two sub-samples.

Determination

The pelleting material and / or tape material shall be removed as described earlier but drying is not obligatory. The working sample is searched wither for seeds of all other species or of certain designated species, as required by the sender.

Calculation and expression of results

The results is expressed as the number of seeds belonging to each designing species of category found in the actual weight and approximate number of pelleted seeds examined and for seed tapes the length of tape (or area of mat) examined. In addition the number per unit weight, per unit length or per unit are (e.g. per kilogram, per metre or per square metre) may be calculated.

Reporting results

The actual weight and approximate number of pelleted seeds and / or the length of tape (or are of mat) examined and the scientific name and number of seeds of each species sought and found in this weight, length or area shall be reported on the International Seed Testing Association International Seed Analysis Certificate under Other Determinations', not withstanding that the result may in addition be expressed in some other way (e.g. number of seeds per kilogram, per metre or per square metre).

1. **Germination test of Pelleted Seeds:**

To determine the percentage by number of normal seedlings of the kind of seed of which the sample purports to be, using pellets from the pure pellet fraction or tape without removing the seeds from the tape material. An additional germination test on pure seed taken of the pellets or tape may be carried out at the request of the sender or as a check on a test of pellets or tapes, but care must be taken that the covering material is removed in such a way as not to affect the germination capacity of the seeds.

A pellet is regarded as having germinated if it produces at least one normal seedling of the species stated by the sender. Seedlings that are obviously not of the species stated by the sender, even if normal for their species, are not included in the germination figure but their number shall be reported separately.

General principles

Germination tests on pelleted seeds shall be made with pellets from the pure pellet fraction of a purity test. The pellets shall be placed on the substrate in the condition in which they are received (e.g. without rinsing or soaking). Germination tests on seed tapes are made on the tape without removing the seeds from the tape material or in any way pre-treating the tape.

Materials

Paper, sand and in certain situations soil are permissible as substrates. For pelleted seed the use of pleated paper, and for seed tapes a between paper method of which the upright rolled towel has proved satisfactory in many cases, is recommended.

The pleated paper recommended for tests on pellets has a weight of 100 – 120 per square metre and water absorption of 220-240%. The pleated filter papers are enveloped by cover strips, of weight 70g per square metre, and water absorption of 220-240%.

Counting equipment *viz.*, counting boards, vacuum counters

Germination apparatus i.e. The bell-jar or Jacobsen apparatus, the germination cabinet, and room germinations.

Procedure

Working sample

The pure shall be well mixed and 400 pellets counted at random in replicates of 100. The working sample from seed tapes shall consist of randomly taken pieces of tape to make up four replicates of at least 100 seeds each.

Test conditions

Methods, substrates, temperatures, light conditions and special treatment as for particular species should be used. Where substrates prescribed are found not to give satisfactory results, pleated paper should be used for pellets and a between paper method for tapes.

Moisture and aeration

The water supply may be varied according to the pelleting material and the kind of seed so as to achieve optimum conditions for germination. If pelleting material adheres to the cotyledons, water may be sprayed cautiously on to the seedlings at the time of counting.

Special treatments for breaking dormancy

When fresh ungerminated seeds remain at the end of the test period a retest may be made using one of the special treatments prescribed.

Duration of test

Extension beyond the period prescribed may be necessary. However, slow germination may be an indication that test conditions are not optimum and a germination test of seeds removed from the covering may be made as a check.

Evaluation

Evaluation of seedlings as normal or abnormal shall be in accordance with the definitions and details of the essential structures. Abnormality may on occasion be due to the pelleting or tape material and when this is suspected a retest shall be carried out in soil of good quality.

Multiple seed structures

Multiple seed structures may occur in pellets or in tapes or more than one seed may be found in a pellet. In either case these shall be tested as single seeds. The result of the test indicates the percentage of structures or pellets which have produced at least one normal seedling. Pellets or seeds in tapes producing two or more such seedlings are counted and their number recorded.

When pellets are tested for monogermity the numbers of pellets which have produced either one, two or more than two normal seedlings are determined in the germination test and each is expressed as a percentage of the total number of pellets producing at least one normal seedling.

Calculations and expression of results

Results expressed as percentage by number. In addition, for taped seeds the total length of tape (or area of mat) used in the germination test is measures and the total number of normal seedlings is noted. From these data the number of normal seedlings per meter (or square metre) is calculated.

Reporting results

The percentage of pellets or seed in tapes with normal seedlings, with abnormal seedlings and without shall be reported on the International Seed Testing Association International Seed Analysis Certificate. The method used for the germination test and the duration of the test must be indicated. In addition, for seed tapes the number of normal seedlings per metre of tape (or square metre of mat) shall be reported.

4. Weight determination and size grading of pelleted seed

Because of the technical requirements of precision drilling, weight determination or size grading may be necessary.

Object

The object is to determine the weight per 1000 pellets and/or size grading of the sample as submitted.

Principles

For a weight determination the number of pellets in a weighted quantity of pure pellets is counted and the weight per 1000 calculated. For size determination a sample of the size specified in Appendix A is screened as specified and the percentage of each screening fraction determined.

Apparatus

For weight determination a suitable counting machine or counting equipment for germination tests may be used. For size determination a suitable screening machine is used.

Certificates

Reporting results

International Seed Testing Association International Seed Analysis Certificates for coated seeds should be clearly marked in the space following the heading ANALYSIS RESULTS with the words 'SEED PELLETS, ENCRUSTED SEEDS, SEED GRANULES, SEED TAPES or SEED MATS'. The name and number of seeds of each species found in the verification of species examination shall be reported under 'Other Determinations'.

*****

NATIONAL SEED RESEARCH & TRAINING CENTRE
G. T. Road, Collectry Farm,
Varanasi- 221106 (U.P.)
Tel: 0542 2370222
E-mail: dir-nsrtc-up@nic.in
Website: www.nsrtc.nic.in