National Workshop

on

SEED TESTING

(March 13-15, 2024)

Training Manual

Organized by:

Government of India
Ministry of Agriculture and Farmers Welfare
Department of Agriculture and Farmers Welfare
National Seed Research and Training Centre, Varanasi

NATIONAL WORKSHOP
ON
SEED TESTING
(MARCH 13-15, 2024)

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FOREWORD

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

It gives me immense pleasure to inform that National Seed Research and Training Centre, Varanasi is organizing the **National Workshop on "Seed Testing"** during **March 13-15, 2024** at NSRTC, Varanasi. This National Workshop is of utmost important to augment the income of farming community and agricultural production through the availability of quality seeds across the country.

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

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

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

(Manoj Kumar)
Director

Date: 15.03.2024

Place: Varanasi

National Workshop
On
Seed Testing
(March 13-15, 2024)

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NSRTC at a glance...

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

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

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

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

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

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

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

PRIME OBJECTIVES:

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

VISION:

Our vision is to

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

MISSION:

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

STRATEGY:

NSRTC pursues its Mission and Goals through

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

Staff strength:

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

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

National Seed Testing Laboratory as Central Seed Testing Laboratory

The testing of seed material will be flowing from different State Seed Corporations as well as Seed Producing Organizations for physical purity, seed health and at later stage genetic purity that is mostly required in referral cases. At present the mandate of Central Seed Testing Laboratory (CSTL) is to receive 5% samples from seed producing organizations all over the country. In addition, CSTL act as a Nodal centre for coordinating the activities of Seed Quality

Control programmes on behalf of Government of India in accordance with the Act and Rules with the State Notified Seed Testing Laboratories.

Grow Out Test

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

NSRTC is geared to go Global

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

- To promote the availability of quality seed to meet the challenges of Science based Agriculture.
- Making of promising Technologies reach the seed entrepreneurs and other stakeholders through innovative Trainings, Conferences, Workshops & 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 Quality Regulatory Regime

An insights and synthesis of regulatory aspects that affect seed trade

Prof. Dr. M. Bhaskaran, Pro Vice Chancellor, VISTAS, Chennai

Introduction

All farmers require high quality seeds of improved varieties for each planting season. The accessibility of a consistent supply of high-quality seed is the crucial to a viable and dynamic agricultural sector. Seeds are a rudimentary input for overall crop cultivation; as such seeds are basic to food and nutrition security. Worthy seed is vital for the rural development and farmers' livelihoods and for all green value chains. Seeds are also important for the means of support tactics of farming communities worldwide. The ongoing efforts to advance new plant varieties and the distribution of these varieties quality seed to farmers across the globe are of paramount importance. The viable accessibility of seed with superior seed choice is thus an essential issue for every farmer, who access seeds from multiple sources. Different seed systems with specific regulations across the regions and countries have their strengths and vulnerabilities. To ensure that adequate supplies of high-quality seed are available to agricultural producers in both the domestic and foreign markets requires a consistent checking of quality at all stages of the supply chain. The many stakeholders along the seed supply chain including breeders, producers, traders, regulators and farmers work together to ensure that quality standards are maintained and, indeed, enhanced. Close coordination of the work in breeding, testing and certification of seed is critical in facilitating trade of high-quality seed and in lowering non-tariff barriers.

With shifting farming conditions around the world due to climate change, soil degradation and market demands, an analysis of possible challenges and strategies future-proofing seems essential for smooth movement and trading of seeds. Such analysis on challenges and strategies also informs the kinds of support that different seed systems may need to be optimally resilient for the betterment of seed industry stakeholders.

A functioning seed quality regime should sustainably allow farmers to have access to the seeds of their choice and purpose, of the best possible qualities, the right time and at the right price from the farmers' investment perspectives by overcoming all the prevailing challenges. All regulatory systems include aspects of breeding, production and distribution in more or less complex settings. These include the corresponding distribution of tasks between multiple operators, including seed policy development and implementation at various levels.

Irrespective of seed quality regulation / assurance, the terms formal and informal seed systems are commonly used to distinguish between seed classifications, however government closely monitors the checks and balances including imposing punitive action against the offenders. For all seeds, farmers will decide their source on the basis of

availability, perceived quality aspects and cost. Resilience of each seed system and regulatory regime is essential for effective regulations not only in the interest of farmers, seed supplier, stakeholders but also for the enforcing agencies to comply with the quality requirements of the market while seed movements and trading at all levels.

Despite several decades of policy reforms, implementation of seed quality regime, decision making on seed systems exits with many challenges. We explores India's rapidly growing seed industry to illustrate current gaps and strategies for used to shape competing narratives and inform current policy discourse around seed system develop in the region for the benefit of the intended beneficiaries at national and global scenario. The trusted high performing seed quality regime should be with right regulations, systems, and processes to support and ensure seed quality and agriculture sector growth and prosperity. There are adequate provisions under existing seed legislations to regulate the quality of seeds. The mechanisms for such regulation are in keeping with the federal structure of the country. The powers of enforcement and implementation are vested in the State Governments.

Seed status and quality regime – An appraisal @ national and global perspective

Domestic and Global seed trade has also been progressively increasing. India which has rich genetic diversity of crops and natural resources has an immense potential for growth for supplying seeds not only to the domestic industry, but also by increasing exports and servicing international markets.

India has varied agro-climatic zones ranging from tropical, sub-tropical and regions with temperate climatic conditions, which provides a unique proposition to realize the potential for becoming a seed production base for market requirements of many countries in the world.

India has strong capabilities in conventional plant breeding; Research and Development which will enable Indian companies develop and supply seed to countries with similar agro-climatic regions in Asia, Africa & other parts of the world.

Decision-making expertise available in both public and private sectors in terms of implementation of national and international quality standards, quality seed production systems, establishment of seed enterprises in accordance with the regulatory frameworks, policy and management of supply chains and distribution systems.

Availability of human resources of skilled and trained manpower in Plant breeding, seed technology, agronomy, horticulture, biotechnology and life sciences available for expansion of seed industry to meet domestic and global seed demand. Capacities to serve both domestic demand, but also create capacities to cater to export demand. A strong regulatory policy and enforcement mechanism for protection of Intellectual property and seed trade and hence Indian seed industry has a potential to be a seed hub not only @ national level but also at globally.

Seed market analysis

In India, since 1965, 5,967 improved field crops varieties have been developed which include 2,943 of cereals, 975 of oilseeds, 1,083 of pulses, 233 of forage crops, 538 of fibre crops, 146 of sugarcane and 49 of potential crops. During 2021–22, a total of 467 varieties/hybrids including 35 special traits varieties (23 varieties developed through marker assisted selection and 12 bio fortified varieties) were notified and released for commercial cultivation (ICAR Annual report 2022-23). 514 l LQ of certified seeds were produced available for distribution over and above the seed target requirements.

The **Indian seed market** size was valued at USD 6.3 Billion in 2022 and is likely to reach USD 18.08 Billion by 2031, expanding at a CAGR of 12.43%. The growth of the market is attributed to the rising demand from the agriculture sector. Indian agriculture has made considerable progress since the green revolution of the last part of the 1960s**.** India presents an intriguing situation, both GDP and food grain creation in the nation have risen quicker than the development in populace throughout the most recent 50 years. Indian seed industry is going through wide change including expanded part of private seed organizations, section of MNCs, and joint endeavours of Indian organizations with worldwide seed organizations.

The value of **Global seed market** size is estimated at USD 66.85 billion in 2023, and is expected to reach USD 92.02 billion by 2028, growing at a CAGR of 6.60% during the forecast period (2023 – 2028 - Source:https://www.mordorintelligence.com/industry/seeds).

Framework for Seed Quality regulation in India

The Central Legislations like the Seeds Act, 1966, the Seed Rules, 1968, the Seeds (Control) Order, 1983 and Environmental (Protection) Act, 1986 have empowered the State Governments to check the quality of seeds and curb the sale of sub-standard/spurious seeds. The responsibility of execution of various provisions of the Central Legislations vested with State government. The Seed Inspectors have been appointed under the Act can inspect the premises of seed selling, marketing, processing and seeds storage to draw the samples for seeds testing to ensure prescribed seeds standards. Appropriate action/legal proceedings may initiate against the offenders selling sub-standard seeds as per the provisions of the Seeds Act/Rules and Seed (Control) Order. These Seed Inspectors are also authorized to stop the sale of sub-standard seeds and to seize their stocks. Seed Inspectors have also been provided with powers of enforcement under the Environment (Protection) Act, 1986 to regulate the quality of Genetically Modified (GM) crops.

By effective implementation of all appropriate legislation's, the multiplication and distribution of quality seeds of all crops are being ensured so that the required quantities of seeds can be made available in time to farmers in the country.

A number of international organisations, conventions and treaties deal with the regulation of seed trade, ranging from access to delivery of quality seeds to growers. Together they provide an international regulatory framework by overseeing the interests of breeders, producers and consumers. Among these organisations, the Organisation for Economic Co-operation and Development (OECD) Seed Schemes are globally recognized for the certification of seed moving in international trade. The International Seed Testing Association (ISTA) developed globally recognised standard procedures for seed sampling and testing. The International Union for the Protection of New Varieties of Plants (UPOV) provides breeders of new plant varieties with an intellectual property protection. Besides, there are the international conventions and treaties the Food and Agriculture Organization of the United Nations (FAO) that provide the international regulatory framework for related aspects of seed trade, including plant health and phyto-sanitary measures, International Treaty on Plant Genetic Resources for Food and Agriculture by access and benefit-sharing for germplasm. An appropriate regulatory framework helps to promote competitive seed markets and lowers barriers to trade. This lead paper discusses the challenges and strategies for effective implementation for the facilitation of making available and movement high quality seeds of improved varieties quality regulatory regime at national and global perspectives.

Network of seed quality control

Quality control activities of seed cover seed certification, seed testing, seed law enforcement and labelling of seeds etc.,

Seed certification: Seed certification is a quality assurance process. Seed intended for domestic or international markets is controlled and inspected by official sources in order to guarantee consistent high quality for consumers. This is done by i) controlling the seed in previous generations ii) carrying out field inspections during the multiplication process to ensure there is little contamination and that the variety is true to type iii) growing samples in control plots of the known seed to ensure that the progeny conform to the characteristics of the variety and iv) seed quality testing in laboratories. Of all the regulatory standards relating to seed laws, certification probably varies the most between different countries and parts of the world. Almost all countries developed their own certification standards (some have accepted the OECD Seed Schemes Rules as their national standards). In some countries certification is obligatory and in others optional. Some certification schemes focus on the genetic characteristics; in others, varietal certification is complemented by tests

carried out in order to check laboratory standards, such as minimum germination, minimum analytical purity and seed health.

Seed testing: Seed testing is the key aspect in determining quality of seeds. Accordingly, Section 4 (2) of the Seeds Act, 1966 empowers the State Government to establish one or more State Seed Testing Laboratories in the State. There are more than 130 laboratories and 2 central laboratories in the country. The central laboratories are NSRTC at Varanasi and the laboratory at CICR Nagpur (for GM cotton only).

Seed tests provide farmers, seed traders and regulators with information on the quality of seed before it is sown. Seed quality can be described as the overall value of the seed, resulting from genetic characteristics and from all other factors affecting the development, maturation and storability of the seed. Seed testing laboratories evaluate these characteristics. Quality attributes tested in seed test laboratories include minimum physical purity and limits on seeds of other crops and weed species; minimum germination; limits on moisture contents and limits on seed-borne diseases. Other quality aspects evaluated by a laboratory may include seed size and weight, seed vigour, seed viability and varietal quality assessment, which may include the detection of genetically modified organisms.

Labelling of seeds

As per the Seeds Act, 1966 and the Seeds (Control) Order, 1983, the labelling of seeds is compulsory for any kind or variety of seeds being sold in the market.

If any person selling seeds without label or giving false information on the label, the seed inspector appointed under the seed legislations can take appropriate actions to curb the supply of such sub-standard/spurious seeds in the market.

Enforcement of Seed Laws

Responsibility for enforcing seed laws is vested with the State Government. State Government appoints seed inspectors for this purpose. Seed inspectors draw the samples from seed lot in the market and send the same to the seed testing laboratories to assess whether the seeds conform to seed standards. In case the results do not conform to the seed standards, they can issue stop sale order. In case of seeds found sub-standard, a case is filed in the trial court. The Seeds Act, 1966 and the Seeds (Control) Order, 1983 imposes fine and imprisonment of the person found guilty.

National Policies:

National seed policy: The policy should facilitate a technical and facilitating role in the development of national seed policies through a participatory process involving the key stakeholders in the public and private sector. It should deal with how the next level

policies can be best used to develop the seed sector: seed quality assurance schemes including seed quality control and variety release procedures; legislation; extension services; manpower development; credit and subsidies; local seed enterprises; international cooperation etc.

Regional harmonization of seed regulations: The Regime should also provide a technical and facilitating role in the harmonization of seed regulations at regional level, which is recognized as vital for the development of the seed sector, because it facilitates cross-border movement of seeds both at national and global level and provides a broader market for seed enterprises. By facilitating cross-border seed trade, the harmonization of seed regulations also allows countries with seed deficits to trade more easily with neighbouring countries with seed surpluses, thereby promoting seed security.

Policy on Export/Import of seeds and planting materials: The export /import of seeds has increased with rationalization and simplification of the export/import regime. This has benefited Indian farmers, the seed industry and entrepreneurs. During the year 2021-22, 313 cases of export and 142 cases of import have been recommended to make available the best seed and planting material to farmers.

GM crops in India: Bt. Cotton is the only genetically modified crop approved for commercial cultivation by Genetic Engineering Appraisal Committee (GEAC) of the Ministry of Environment, Forests & the Climate Change (MoEF&CC) in the major cotton growing States viz. Andhra Pradesh, Telangana, Tamil Nadu, Karnataka, Maharashtra, Madhya Pradesh, Gujarat, Rajasthan, Haryana and Punjab.

Seed price regulation

There is no provision for regulating price of seeds under the Seeds Act 1966, the Seed Rules, 1968 or the Seeds (Control) Order, 1983. Seed is an essential commodity in terms of Seed (Control) Order, 1983, for the purpose of regulating the quality of seed only, the price of seed is not fixed/regulated.

GM price regulation: In order to provide an effective system for fixation of a uniform maximum price and to ensure the availability of Bt. Cotton hybrid seeds to farmers at fair, reasonable and affordable prices, the Government of India issued a Cotton Seeds Price (Control) Order (CSPCO), 2015 on 07.12.2015 under section 3 of the Essential Commodities Act, 1955. Accordingly, the Government has regulated the sale of Bt. Cotton seed by evoking the provisions of the EC Act.

Boosting Seed Production in the Private Sector: Under this component of credit linked back ended subsidy, @40% of the capital cost of the project in general areas and 50% in case of hilly and scheduled areas subject to an upper limit of Rs 150 lakhs per project is funded. Two percent (2%) of the total fund utilized under the component is provided as administrative charges to the nodal agency.

Policy initiatives taken to enhance production and distribution of quality seeds

The steps taken includes:

1. Systematic production of Breeder, Foundation and Certified/Quality seeds
2. To provide assistance under different schemes being implemented by the Department of Agriculture, Cooperation& FW, GOI through the State Governments for seed production and distribution, creation of infrastructure for storage, processing, laboratories, quality control, capacity building etc.,
3. Special intervention to improve quality of farmer saved seeds under Seed Village programme.
4. Formulation of policies/plans viz. New Policy on Seed Development, 1988; National Seed Policy 2002 , National Seed Plan 2005-06 and Seed Act 1966/Seed Rules/Seed Control Order, to ensure an overall legal and regulatory framework for availability of seeds.
5. Enhancing seed production through public sector seed producing agencies viznational level Corporations (NSC) and 23 State Seed Corporations
6. Facilitating varietal replacement rate
7. Under the existing import policy relating to seeds, the best planting material available in the world is allowed for import by private/public companies subject to quarantine regulations.
8. 100% FDI is allowed under the automatic route for development and production of seeds and planting material subject to certain conditions.

Modifications in new policy on Seed Development: The National Seed Policy, 2002 provides that all imports of seeds and planting materials etc. promoted subject to EXIM Policy Guidelines and the requirements of the Plants, Fruits and Seeds (Regulation of import into India) Order, 1989 and amendments thereon. The import of parental lines of newly developed varieties will also be encouraged. The policy also ensure that imported seeds and planting materials should meet the minimum seed standards of seed health, germination, genetic and physical purity as prescribed.

All importers will make available a small sample of the imported seed to the Gene Bank maintained by the National Bureau of Plant Genetic Resources (NBPGR). In order to harmonize the New Policy on Seed Development, 1988 with the National Seed Policy, 2002,

the following two modifications have been made in the new Policy on Seed Development, 1988.

(i) Seeds of wheat and paddy – In order to provide to the Indian farmer the best planting material available in the world to increase productivity, the import of seeds of wheat and paddy may also be allowed as per provisions of the Plant Quarantine Order, 2003 as amended from time to time for a period not exceeding two years by companies, which have technical/financial collaboration agreement for production of seeds with companies abroad, provided the foreign supplier agrees to supply parent line seeds/nucleus or breeder seeds/ technology to the Indian company with in a period of two years from the date of import of the first commercial consignment after its import has been recommended by DAC. For trial and evaluation of the variety sought to be imported by eligible importers, 16 kg of seed in case of wheat and 5 kg in case of paddy will be given to ICAR or farms accredited by ICAR for sowing. After receipt of satisfactory results of trial/evaluation, an eligible importer may apply for bulk import of such seeds to the Department of Agriculture and Farmers' Welfare.

(ii) Similar procedure of trial/evaluation has been recommended for import of seeds of coarse cereals, pulses and oil seeds. Further, Seeds Division has simplified the forms for export and import of seeds and planting material.

(iii)

Enactment of Protection of Plant Varieties and farmers' Rights Act

Government of India ratified the Agreement on Trade Related Aspects of Intellectual Property Rights, accordingly, passed the **Protection of Plant Varieties and Farmers' Rights Act,** 2001 in Indian Parliament to establish an effective system of protection of plant varieties and farmers' rights in harmonisation of the UPOV system of Novelty, Distinctness, Uniformity and Stability. Consequent upon the enactment of **Protection of Plant Varieties and Farmers' Rights Act, the rules were framed in 2003.**

Protection of Plant Varieties and Farmers' Rights Authority (PPV&FRA): Implementation of PPV&FRA, the DAC&FW provides administrative support to the Statutory Organization Protection of Plant Varieties and Farmers' Rights Authority functioning as per the provisions of the Protection of Plant Varieties and Farmers' Rights Act in 2001. The Authority was set up in 2005. In all proceeding under this act, the Authority or the Registrar, as the case may be, shall have all the powers of a civil court for the purposes of receiving evidence, administering oaths, enforcing the attendance of witnesses, compelling the discovery and production of documents and issuing commissions for the examination of witnesses and any such order shall be executable as a decree of a civil court.

Implementation of OECD Seed schemes in India: The objective of the Organization for Economic Co-operation and Development (OECD) Seed Schemes is to encourage the use of seeds of consistently high quality in participating countries. The scheme authorizes the use of labels and certificates for seeds produced and processed for international trade according to OECD guidelines. The scheme also facilitates the import and export of seeds, by the removal of technical trade barriers through internationally recognized labels. India's participation in OECD schemes aims to enhance seed export capabilities and probabilities. The Department of Agriculture & Farmers Welfare has become a member of the OECD Seed Scheme from 23 October, 2008 and 260 varieties in more than 20 crops have been offered for enlistment in OECD list of varieties since the inception of the scheme. A National Task Force on OECD Scheme has also been constituted to enhance India's share of seed export through this scheme.

Provisions under existing legal instruments - The Seeds Act, 1966 and the Seed Rules, 1968.

When the variety is notified, such variety sold in the market it should be labelled as prescribed under section 6(a) deals with the standard of germination and physical purity and 6(b) deals with colour of the label, content of the label, size of the label of the Seeds Act, 1966. If any person contravenes any provision of the Seeds Act, 1966, the Seed Inspector notified under section 13 of the Act empower to prosecute him for which the fine which may extend to Rs. 500 for the first offense and it is repeated for which the fine may extended to Rs. 1000/- or imprisonment for six months are with both.

The Government of India notified Central Institute of Cotton Research (CICR) Nagpur as Central Seed Testing Laboratory under section 4 (I) of the Seeds Act, 1966 to test all events of Bt. gene samples which are referred by the Court of Law for presence or absence of Bt. gene and its strength for which the standards are prescribed. It is learnt that some of the company notified their cotton varieties under section 5 of the Seeds Act, 1966 for the first instance and then later on the incorporated the Bt. gene in their cotton hybrid for example cotton hybrid c.v. Malika and Bunny.

The Seeds (Control) Order, 1983: The Seeds (Control) Order has been issued in exercise of the powers conferred by section 3 of the Essential Commodities Act, 1955. As far as standard is concerned the standards notified under section 6 of the Seed Act is equally applicable to the Seed which are regulated under the Seed (Control) Order, 1983. As per the penalty is concerned it is to state the penalty is applicable under the EC Act is equally applicable to this order.

Seed inspectors appointed under Section 12 of the Seed (Control) Order empowered to draw the samples of seed, whether it is notified or non-notified under section 13 of the said order, meant for sale, export and import, and sent the dame in accordance with the procedure laid down to a laboratory notified under the seeds Act. To ensure that the

samples confirms to the standards notified under section 6 of the Seed Act therefore, the GM Crops which are mostly non notified covered under the provisions of the Seeds (Control) Order, 1983.

The Environment (Protection) Act, 1986

The Environment (Protection) Act, 1986 empower the seed inspectors, seed testing laboratories notified under relevant provision of the seed Act to draw the seed sampled of GM Crop whether notified or non-notified and to send the samples to the laboratory to confirm the standards prescribed under the Seeds Act, 1966 and the Seeds (Control) Order.

Challenges and Salient features of the Central Legislations: Seeds Act, 1966 and the Seed Rules, 1968.

i. The Seeds Act/Rules are applicable to notified crop variety seeds.
ii. Notification of kind/varieties of seeds under Section 5 of the Act.
iii. As per section 6 of the Act, minimum limits for germination, physical and genetic purity of varieties/hybrids for crops have been prescribed
iv. Labelling of seed is compulsory as per the section 7 of the Seeds Act.
v. State Governments have powers to appoint Seed Analysts and Seed Inspectors under section 12 and 13 of the Act respectively.
vi. Seed Inspectors are vested with adequate powers for quality control viz. to draw the sample; enter and search; examine records, registers, and documents; seize the stock and issue 'Stop Sale' order in case the commodities under reference contravene provisions of law under the section14 of the Act.
vii. As per the sub-section 5 of the section 14 of the Act, Inspectors are authorized to take punitive action/ launch proceedings against dealers found to be selling sub-standard seeds.
viii. The seed in respect of which the contravention has been committed can be forfeited under Section 20 of the Seeds Act.
ix. Penalties are provided under Section 19 of the Act i.e. a fine of Rs. 500 for the first offence (if repeated, the fine may be extended to Rs. 1000/-) or imprisonment for six months or with both.
x. Provision made in the section 9 of the Act for seed certification of notified kind/varieties to ensure genetic identity and purity. Certification includes the Indian Minimum Seed Standards and Seed Certification procedures.
xi. As per the section 17 of the Act, export or import of seed of any notified kind or variety is allowed subject to conditions such as conforming to the minimum limits of germination and physical purity, etc.

Seeds (Control) Order, 1983

i. The Seeds (Control) Order is applicable to both notified and non- notified kind of seeds.
ii. As per the Clause 12 of the Order, State Governments have powers to appoint Seed Inspectors.
iii. Seed Inspectors are vested with adequate powers under the clause 13(1) of the Order for quality control.
iv. As per clause 13 of Order, Inspectors are authorized to take punitive action/ launch proceedings against dealers found to be selling sub-standard seeds.
v. The business of selling, exporting and importing seeds can be carried out only under a licence issued by the State Government under the provisions of clause 3 of the Order.
vi. A dealer's license is liable to be suspended/cancelled for contravention.
vii. As per clause 8 of Order, seed dealers are required to maintain books and accounts and display the stock position and its price.
viii. The dealers can also be directed to distribute seeds in specified manner in public interest under the provision of clause 10 of the Order.
ix. Joint Secretary Seeds), Department of Agriculture and Cooperation&FW has been declared as **Seed Controller of India** under the provision of clause 10 of the Order.
x. The penalties are provided under the section 3 of the Essential Commodities Act, 1955.

Concern: There is no clarity exists in consistent implementation among states.

Salient features of Environmental Protection Act, 1986

The Ministry of Environment & Forests empowered the Seed Inspector notified Under Section-13 of the Seeds Act and under Clause 12 of the Seeds (Control) Order to draw the seed samples of transgenic seeds as mentioned under Section-10 of EPA, 1986 and to test in the notified seed testing laboratories in case of sale of spurious Bt. cotton seed. Ministry of Agriculture has prescribed minimum limits of purity in respect of Bt. cotton seed (as Bt. Protein- Toxin) under Section-6 of the Seeds Act and notified the laboratory of Central Institute of Cotton Research, Nagpur as referral laboratory for testing Bt cotton seeds.

Bt cotton seed testing

Department of Agriculture and Cooperation has prescribed minimum limits of purity in respect of Bt cotton seeds i.e. presence of minimum limits of 90 % lethal protein in the samples under Section 6 of the Seeds Act, 1966 and prescribed the testing procedure/protocol to test Bt cotton seeds. The Government of India notified Central Institute of Cotton Research (CICR) Nagpur as the Central Seed Testing Laboratory under

section 4 (1) of the Seeds Act, 1966 to test all events of Bt gene samples which are referred by the Court of Law for presence or absence of Bt gene and its strength for which the standards are prescribed.

ELISA kit (Bt Quant) Technique has been prescribed by Department of Agriculture and Cooperation& FW in consultation with Indian Council of Agriculture Research to detect the presence or absence of specific event in the Bt cotton seed/plants.

Exiting quality enforcement measures for GM Seeds

The Ministry of Environment & Forests empowered the Seed Inspector notified Under Section-13 of the Seeds Act and 12 of the Seeds (Control) Order to draw the seed samples of transgenic seeds as mentioned under Section-10 of EPA, 1986 and to test in the notified seed testing laboratories in case of sale of spurious Bt. cotton seed. Ministry of Agriculture has prescribed minimum limits of purity in respect of Bt. cotton seed (as Bt. Protein- Toxin) under Section-6 of the Seeds Act and notified the laboratory of Central Institute of Cotton Research, Nagpur as referral laboratory for test the Bt. cotton seeds.

Role of Central and State Governments in meeting seed demands.

As Agriculture is a 'State' subject, states are primarily responsible to ensure production, availability and distribution of certified seeds. They make arrangement of seeds through agencies, like, State Agricultural Department, State Seed Corporation, State Agricultural Universities, State Cooperative Agencies and Private seed companies. National agencies like NSC, SSCs etc. are supplementing the efforts of the Government in making available seeds. The Government of India supports the efforts of the State Governments by coordinating the position of seed requirement and availability well in advance. The Central Government also provides assistance to the State Government and Seed Producing Agencies for enhancing production and distribution of seeds under various programmes.

Bio-safety regulations in India

The Ministry of Environment & Forests (MoEF) had enacted Environment (Protection) Act in 1986 to provide for the protection and improvement of environment and the related matters. Environment includes water, air and land and the interrelationship, which exists among and between water, air and land, and human beings, other living creatures, plants, microorganism and property.

Rule, 1989 under Environment (Protection) Act, 1986

MoEF has notified the "Rules for the Manufacture, Use/ Import/ Export and storage of Hazardous Micro Organisms/ Genetically Engineered Organisms or Cells through Notification No. 621 in the Gazette of India on December 5, 1989 under the provisions of

the EPA with a view ensure sound application of biotechnology making it possible to accrue benefits arising from modern biotechnology while minimizing the risks to environment and human health.

These rules and regulations commonly referred as 'Rules1989' cover areas of research as well as large scale applications of GMOs and their products including experimental field trials and seed production. Rules 1989 is supported by the following bio-safety guidelines: which have been developed through a consultative approach and following the international norms prescribed by the Organization for Economic Co-operation and Development (OECD), CODEX, Aliment Arius, Commission and International Plant Protection Convention (IPPC).

- Recombinant DNA Safety Guidelines, 1990 & 1994
- Revised Guidelines for research in transgenic plants and guidelines for toxicity and Allergen city Evaluation, 1998
- Guidelines and SOPs for the conduct of confirmed field Trials of Transgenic Plant, 2008
- Guidelines for the Safety Assessment of GM Foods, 2008
- Protocol for Safety Assessment of Genetically Engineered Plants/ crops, 2008

Rules 1989 is implemented by MoEF and Department of Biotechnology (DBT), Ministry of Science and Technology. MoEF is primarily responsible for conservation and protection of environment, ensuring environmental and human health safety before release of genetically modified organisms (GMOs) and products thereof. The mandate of the DBT is to promote biotechnology, provide services in areas of research, infrastructure, generation of human resource and formulation of guidelines and protocols for safety assessment of GMOs.

Challenges in the current regulations:

During the year 1966, in the interest of the increased agricultural production in India, it was considered necessary to regulate the quality of certain seeds such as seeds for food crops, cotton seeds and Jute seeds to be sold for the purpose of agriculture including horticulture. It was, therefore, considered necessary to have legislation for regulating the quality of certain seeds and accordingly the Seeds Act, 1966 was enacted. However, during the working of the said Act for more than four decades, the following deficiencies have been noted:-

i. The Seeds Act, 1966 regulates only notified kind and varieties and does not address non-notified varieties, research/private hybrids and varieties, etc.;

ii. Registration of seeds of kind/variety, seed producer, seed processing unit and seed dealer is not compulsory;

iii. Green manure seeds, narcotics, plantation crops, medicinal and aromatic plants, etc. are not covered under the Act;

iv. No provision for regulation of transgenic materials and no prohibition of terminator technology, etc.

v. No provision for regulation of fruit nursery;

vi. The penalties for contravention of the provisions of the Act are very mild and inadequate for any deterrent effect;

vii. The provisions for exempting the sale of seed for purposes other than for sowing or planting are ambiguous;

viii. No provision for regulation of dealer, distributor, seed processing units, etc.

ix. No provision for compensation to the farmer in case of seed failure;

x. No provision for regulation of seed price even in the emergent situations;

xi. No provision for accreditation of private organizations for evaluation of performance;

xii. No definition for farmer & producer;

xiii. No provision for regulating the seed health quality.

Necessity for new legislations

During the last five decades, seed production technologies have changed and new technologies like transgenic, tissue culture, soil-less agriculture etc. have emerged. There is greater emphasis on seed quality assurance particularly to safeguard the interest of the farmers. There has also been a significant change in the country's socio-economic conditions. The economy has been substantially liberalised and the private sector is playing an increasing role in various spheres including agriculture. India is also integrating with the rest of the world through the World Trade Organization (WTO), Organization for Economic Co-operation and Development (OECD), International Seed Testing Association (ISTA), and International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), etc. The above necessitates up-gradation of seed production, quality and regulatory standards. Therefore, a need has arisen for up-scaling the seed quality regime by enacting a new legislation.

Scope of new legislation to strengthen regulation of seed quality

i) **Compulsory registration of National varieties by the Registration Sub-Committee and State Varieties by State Seed Committee**. No person will be allowed to carry on the business of selling or supplying any seed which is not of a registered kind/variety. Farmers are to be exempted from compulsory registration. Registration will be for a fixed period but can be pre-maturely cancelled for stated reasons.

ii) **Enhancement of penalties** is proposed from Rs.500 / Rs.1000 with or without imprisonment in the Seeds Act 1966 to Rs.25, 000/ Rs.5, 00,000 with or without imprisonment.

iii) **Provision for labelling of expected performance** of seeds has been included so that the farmers are assured of quality of seeds purchased by them.

iv) Provision for **compensation to the farmer** in case of seed failure has been made.

v) **Seed price regulation** of State varieties by the State Government and national varieties by the Central Government under emergent situations involving *inter-alia* scarcity of particular seed varieties, abnormal rise in prices or monopolistic pricing/profiteering.

vi) The Bill proposes to emphasise the **rights of the Farmers** in conformity with the Protection of Plant Varieties and Farmers' Rights Act, (PPV & FR Act) 2001. The farmer will have the right to grow, sow, re-sow, save, use, exchange, and share or sell his farm seeds and planting materials except when he sells such seed or planting material under a brand name.

vii) The Government will have the **right to exclude certain kind or variety of seeds from registration** to protect public order or public morality or human, animal and plant health or to avoid serious prejudice to the environment.

viii) **Seed health** has been added as an additional standard for quality seed.

ix) Provisions to **regulate import and export of seeds** have been largely incorporated into the Seeds Bill itself as given below:-

a) All import of seed shall be subject to Plant Quarantine (Regulation of Import into India) Order, 2003 and other relevant Acts.

b) All imported seeds shall conform to minimum standards of seed health in addition to other conditions already in existence.

c) All imported seeds shall be subject to registration on the basis of information furnished by the importer on the basis of multi-location trials.

d) Exports can be restricted if such exports adversely affect the food security of the country.

x) **GURT (Restriction technology including terminator technology) has been prohibited.** Any person intending to import seed or planting material will declare that such material is, or is not, as the case may be a product of transgenic manipulation or involves Genetic Use Restriction Technology.

The need of hour is to focus simplification of procedures and placing a more efficient regulatory mechanism since;

a. India is emerging as a hub of seed production.
b. Given the growth of the seed sector in recent years, India has the potential to become the foremost player in the seed export business in the developing world with prospective markets in Asia, Africa and South America.
c. But India's present share is less than 2% in the international market. Europe, North America and Asia account for almost four-fifths of the global seed trade.

Current status and Challenges in seed quality regime @ International perspective:

Organization for Economic Co-operation and Development (OECD) Seed Schemes, France and Association of Official Seed Analysts (AOSA), USA are imperative for setting standards and regulations to provide enabling environment for seed industry in India to be competitive with international seed trade. Seed quality assurance is a systematic and planned mechanism for ensuring the genetic, physical, physiological purity and health of seed. It is dynamic with emerging needs of global standards, including land requirement, field crop inspection, seed testing, pre- and post-control.

Several international bodies such as ISTA in 1924 in Switzerland; the International Plant Protection Convention (IPPC) in 1951 in Italy; the OECD Seed Schemes in 1953 in France and the International Union for the Protection of New Varieties of Plants (UPOV) in 1961 in Switzerland were created for setting standards and regulations that provided an enabling environment for the seed industry.

Growth in the international seed trade is being driven by several factors including the rapid decline in transport costs, differential production costs of high-yielding hybrid varieties, better communications and information on the availability of varieties, changing climatic conditions, counter-cyclical production, as well as a more reliable and supportive system of regulations. In general, the seed trade is one of the most regulated sectors in all countries, with a plethora of seed laws, testing and certification procedures. The simplification and harmonization of testing and certification procedures helps to improve farmers' access to high-quality seed in all regions of the world. In many countries seed certification is done at both national and international levels. The most widely used global certification systems are the OECD Schemes, while at the regional level other schemes are used, e.g. EU, AOSCA, etc.

Trade in seed is subject to bilateral and/ multilateral agreements at local, regional, and international levels. As the first input in the cropping process, high-quality seed brings high genetic yield potential resulting in higher productivity and crop production. The regulation authority in charge of seed quality control in most countries is the National Designated Authority (NDA), which has responsibility to ensure the seed meets all the required standards for certification. The OECD Seed Certification Schemes are based on two key criteria; varietal identity and varietal purity.

Varietal identity: The identity of a variety is defined by the official description of its characteristics, resulting from a given genotype or combination of genotypes. Varietal purity: The purity of a variety is the proportion of plants or seeds within the population that conforms to the official description of the variety.

The harmonization of certification procedures at international level has made a significant contribution to developing the global seed trade. The benefits arising from the facilitation of trade in seeds and the improvement in market access are numerous and can be brief as: A lowering of the technical barriers to trade (TBT). The use of worldwide recognized official seed labels and certificates facilitate the exchange of technical information on seed. Encourages the development of seed production in other regions and countries. Contributes to the elaboration of international rules for seed certification. Promotes collaboration between the public and private sectors. Shares experiences and information on emerging issues and concerns in the seed sector. A large number of countries are already participants in the trading and this number is likely to increase as more countries are entering international markets, and seed "consumers" are becoming more demanding with respect to supply consistency, quality and safety. Good cooperation between countries and all stakeholders including international organizations is a response to the need to develop a market-responsive regulatory approach. Every country will continue to be faced with a different legal system and institutional structure and, yet, must compete on the global market.

The rapid growth in the volume of national and International trade of seeds has given rise to many challenges, not least of which is the need to harmonize certification procedures and to adopt reliable and enforceable standards. The OECD Seed Certification System is the most widely used global certification system for the export and import of high-quality seed. The adoption of international certification standards has encouraged the growth in the seed trade by reducing technical barriers to trade, increasing transparency, lowering transaction costs and increasing access by farmers in all regions of the world to high-quality seed.

ISTA produces internationally agreed rules for seed sampling and testing, accredits laboratories, promotes research, provides international seed analysis certificates and disseminates knowledge in seed science and technology. This ensures seed quality and facilitates national and international seed trade, and also contributes to food security. ISTA

provides testing services for companies trading seed internationally. North American countries follow the Association of Official Seed Analysts (AOSA) rules, especially adapted for their market which, however, differs only in minor aspects from that of the ISTA. ISTA and the AOSA have a joint committee on the harmonization of rules. The OECD Seed Schemes provide a system for the assurance of varietal purity and identity for international seed trade, and are normally used in conjunction with ISTA seed lot certificates.

Large scale seed production requires quality control throughout the seed-production processes. International linkages in breeding and seeds require effective phyto-sanitary services. The varietal identity has to be maintained, and seed qualities such as germination percentage and seed health need checking. The quality management required for a reliable label involves independent inspectors and laboratories with significant logistics, facilities and human resources. Poorly functioning systems result in sub-standard seeds entering the market. This may even invite fake seeds, grain in nice bags sometimes with a lemonade colouring to mimic treated seed, creating significant damage to farmers. The most sustainable way is to charge the seed producer the cost of such operations, but this challenges the economic viability of seed production of major food crops and thus defies the objective. Introducing a quality declared seed class can reduce costs especially for remote seed producers with a limited geographical attention, such as farmer–seed cooperatives.

Plant Variety Protection: New varieties of plants with improved characteristics – such as increased yield potential or disease and drought tolerance traits – improve and diversify agricultural production. There are various ways to protect new varieties, including plant variety protection and patents. The WTO's Agreement on Trade Related Aspects of Intellectual Property Rights (TRIPs) obliges its signatories to implement a system for the protection of plant varieties through patents, an effective sui generis system or a combination of both.

Exchange of germplasm and the International Treaty on Plant Genetic Resources for Food and Agriculture: The International Treaty on Plant Genetic Resources for Food and Agriculture is an international legally binding instrument governing access and benefit-sharing for plant genetic resources for food and agriculture (PGRFA). The objectives of the International Treaty are the conservation and sustainable use of PGRFA and the fair and equitable sharing of benefits arising out of their use – in harmony with the Convention on Biological Diversity – for sustainable agriculture and food security. It was negotiated within the Food and Agriculture Organization of the United Nations with the main goals of facilitating the exchange of plant genetic material for research and breeding and of balancing the interests of all involved stakeholders, including commercial breeders and farmers. It came into force in June 2004. By agreeing to make available to each other the

Contracting Parties to the International Treaty established a global gene pool, namely the Multilateral System of Access and Benefit-sharing (the MLS). Facilitated access to PGRFA in the gene pool is accorded through a standard material transfer agreement (SMTA), adopted in 2006 and currently utilised on a global scale.

An effective **phytosanitary control authority** is a prerequisite for all international trade of (agricultural) products. The same capacity is needed to sustain international relations in breeding and seed markets. For variety release and seed quality control, the requirement is more specifically focused on the seed sector. The cost of full seed certification and testing can be quite high, both for the public budget and for the seed sector itself. Accreditation is one way to reduce cost, i.e., provide certain tasks to operators in the district or even to the larger seed producers who have the trained human resources under close supervision by the authorities. Whatever quality management system is chosen, there is a joint interest by governments, stakeholders, farmers and the seed sector to fight fake seed in the market.

Phyto-sanitary measures: Phyto-sanitary measures are government legislations, regulations and procedures that regulate, restrict or prevent the import and marketing of certain plant species or plant products. These measures aim to prevent the introduction and spread of plant pests across international boundaries or to limit the economic impact of regulated non-quarantine pests.

Sanitary and Phyto-sanitary Agreement (SPS).The movement of seed in international trade is based on the principles laid down by the Agreement on the application of Sanitary and Phyto-sanitary Measures (WTO-SPS Agreement) of the World Trade Organization (WTO), agreed in January 1995. The agreement allows countries to set their own phyto-sanitary measures, but these must have a sound scientific basis. The WTO-SPS agreement "restricts the use of unjustified sanitary and phyto-sanitary (SPS) measures for the purpose of trade protection. The aim of the agreement is to maintain the sovereign right of any government to provide the level of health protection it deems appropriate, but to ensure that these sovereign rights are not misused and do not result in unnecessary barriers to international trade. The WTO-SPS agreement also "encourages governments to establish national SPS measures consistent with international standards, guidelines and recommendations. International standards can be higher than the national standards of some countries, but the SPS Agreement allows governments to decide whether they use national or international standards.

Harmonisation of protocols for the facilitation in Seed Trade – Domestic and International Phyto-sanitory measures:

Both the domestic and international movement of seeds as a commodity for seeds for planting or intended for planting supports food production and hunger alleviation at regional, national and globally. Seeds are considered high-risk material in international trade, providing a ready pathway for movement of pests, especially seed-borne pathogens.

The purpose of the International Plant Protection Convention (IPPC) is to prevent the spread and introduction of pests of plants and plant products and to promote appropriate measures for their control. The International Standards for Phyto-sanitary Measures (ISPMs) published by the IPPC provide guidance regarding phyto-sanitary measures and their application to the international seed trade.

Network and coordination of phyto-sanitary regulations: The International Plant Protection Convention (IPPC) is currently recognized by the WTO-SPS Agreement as the only international standard setting body for plant health. These standards are used to harmonise phyto-sanitary measures worldwide with the aim to prevent the introduction and spread of pests of plants, plant products and other regulated articles and to promote appropriate measures for their control. Currently there are nine Regional Plant Protection Organizations (RPPOs) worldwide that facilitate the international harmonization of national phyto-sanitary measures and collaborate with the IPPC. In the 27 Member States of the EU, one main plant health Directive sets out the measures against the introduction of organisms harmful to plants or plant products and against their spread within the EU. In addition, various EU Directives on the marketing of seed and plant propagating material include certain plant health requirements.

Seed testing: **Methods for assessing seed quality**: In agriculture, and more generally in plant biology, where biodiversity is a fundamental element of development, talk of harmonization may seem an absurdity. So why is it so important to harmonize and standardize the methods of quality control of seed? Would it not be simpler and less expensive to let the competition follow its own paths? In terms of quality seeds, what is expected from farmers, the seed trade and regulatory bodies is seed that produces a healthy crop at a fair price. The value of the global seed market is modest, but this trade has an important role in the overall seed industry. In a competitive market it must be possible for the buyer or user to compare the quality of available seeds. To facilitate this, seed quality control must deliver in good time essential information on seed lots. Methods of assessing the quality of seed should therefore be regarded more as measuring tools for industry players, rather than as elements of competition. Competition is related to the quality of seed not to the method of quality measurement.

Harmonization of ISTA testing methods for seed testing quality: The main methods of testing the quality of seeds and their applications: The pillars of seed quality control tests used regularly for trade are, Analytical purity, other seed determination, germination and moisture content tests. Since the 1960s, diagnostic seed-health tests and more recently tests for the detection of GMOs have expanded considerably. Other laboratory tests, such as vigor, tetrazolium viability and varietal purity, are used less for the trade of seed. They are

mainly developed in order to provide information on the performance of seed lots or on their conservation and storage.

ISTA has an international reputation: Its members are drawn from analytical or research laboratories in the public sector as well as from the seed industry. This position at the interface between research, industry and regulation greatly facilitates the identification of needs for new methods or changes in existing methods. In addition, the strong participation of government representatives as members appointed by the designated authorities, and close contacts with international organizations (such as FAO and the OECD) and organizations in specific regions of the world (such as African or Asian bodies) play a major role in the strategic development of ISTA and its methods. The emergence of new regulations, such as the control of GMOs, phyto-sanitary requirements and health, are carefully considered and taken into account. The industry and its representatives at the global level (ISF) or at regional levels demand analytical methods to meet trade requirements and to control risks related to quality. Here the need for detection methods for GMOs is in everyone's mind as is the sanitary quality of seeds, especially among vegetable species where it is a major criterion. The partnership built between the ISTA and the ISHI/ISF (International Seed Health Initiative) is based on their complementary skill sets. The methods in the ISTA rules are there to either meet regulatory needs (e.g. purity, germination, phyto-sanitary) or to satisfy technical and commercial evaluation objectives regarding the potential of seeds (e.g. vigour, state of health).

The technique authentication database: A guarantee for transparency, relevance and traceability to meet the expectations of the trade, methods of quality evaluation must be robust, repeatable and reliable whatever the region where the analysis is made.

Communicating the results in a standard and comprehensive way: The Orange and Blue International Certificates. At the international level they are generally on ISTA certificates (orange for lots of seed, or blue for seed samples) and these certificates are used for import/export transactions. ISTA International Certificates with their ISTA logo guarantee the identity of the seed lot with a single reference; the traceability of the analysis; the competence of the laboratory that made the analysis; the use of referenced methods and standard units; the use of standard reporting languages (English, Latin and others). Today, the ISTA Orange International Certificate (OIC) is widely used for international trade. This is the identity card of seed lots, the pass at many borders and the technical and administrative requirement of many contracts. The OIC is at the top of a pyramid consisting of a set of processes and rules that guarantee the value of the results and form the link between these and a seed lot consignment.

Key strategies: Modernize the Seed Certification System to more effectively support the wide range of seed production business models. Discover and advance approaches that

better integrate the pre and post-harvest elements of the seed certification process. Explore and progress quality management and associated risk based audit system approaches to support the application of models that integrate oversight of pre and post-harvest elements of the seed certification process. Explore and change methods for incorporating seed grower professional recognition programs into any new seed certification system options; single window electronic certification system options to support seed certification system modernization. An exclusive system performance review & improvement cycle and develop with its government and industry partners a meaningful and measurable set of performance indicators for the effective seed quality regulatory systems, with a view to achieving predictably consistent outcomes for the benefits of every stakeholders over time. Establish an annual process to enact improvements to system administration in order to improve performance of central and global legislations with an aim of enhanced output quality through regulatory mechanisms implementations.

Set pest and disease alerts to prevent seed wastage–a significant advantage as research indicates that over 20-40% of global crop production is lost to pests annually. Significantly close monitoring of Pest Risk Analysis from across the production centre / region and appropriate timely decisions (PRA) to fulfil the standards.

Together provide a domestic / international regulatory framework by overseeing the interests of breeders, producers and consumers. Ensuring the right conditions for producing high-quality, a disease-free seed with varietal identity and purity is dependent on managing all critical factors during the production cycle and regulatory stages.

Challenges and for seed companies and stake holders: Key for efficiency and effectiveness: be prepared to tackle with the respective legislations while trading.

i. Harmonization of seed certification procedures in different countries in line with OECD schemes and ISTA procedures or similar global/regional standard.
ii. Standardization of variety registration/commercial release processes, due to varying standards/capacities for VCU testing in different countries.
iii. Standardization of trade and quarantine processes across different regions, trade areas, countries to overcome technical entry barriers.
iv. Chances of delay in custom clearances deteriorate seed viability and quality due to interpretation of regulatory procedures.
v. Capacity building of seed certification organizations at State level in terms of training and human resource development to undertake certification rules and directions as per OECD seed schemes.
vi. Need for capacity development for Plant quarantine facilities, seed testing and certification in a multi-lateral framework.
vii. Issues relating to R&D collaborations and international tie-ups for enabling international seed trade in line with Biodiversity Act.

viii. Fast track clearance for international seed trade through standardization of SPS processes keeping in view seed viability issues.
ix. Counsel and facilitate development, as per gap analysis, of national seed legislation, regulation and policy in line with CBD, ITPGFRA, Nagoya protocols.
x. Development of requisite SPS and bio-safety systems to restrict movement of diseases and pests between countries and to monitor the PRA status.

Emphasis of regulatory regime

i. Uniformity in varietal registration for commercial production and distribution, issuance of license in all states among the stakeholders.
ii. Germplasm movement in most of the countries is through National Bureau of Plant Genetic Resources following phyto-sanitary rules. Vegetables seeds are allowed over phyto-sanitary certificates and import permits. Companies to company's agreement of testing at multi locations and after ascertaining its suitability, the products can be introduced. Company is responsible for its performance and suitability in a particular environment.
iii. Government to promote network of ISTA accredited Seed testing laboratories integrated with OECD schemes (Seed Health Information Systems) through PPP. More sustainable seed business with strong seed systems, processes and business plans with effective regulations as followed by most of the MNC's.
iv. In order to enhance the export of seeds and planting materials and also for sustaining the Indian seed sector, seeds and planting materials should be declared as Normally Traded Commodities under Section 40 of the Biological Diversity Act. 2002. MoEF&CC may be taken care of this issue in the Regulation/ Guidelines.
v. Seed is a germplasm and as such is a carrier of genetic information and biological resource as per section 2 (c) of the BD Act. If the seed of any species has to be exempted under section 40 of the Act, is to be approved by MOA&FW.
vi. The varieties registered under Protection of Plant Varieties & Farmers' Rights Act, 2001 are totally exempted from the applicability of all the provisions of Biological Diversity Act as long as these varieties are used as source of seed or seeding material for use in any commercial activity. This may be cleared by incorporating in the preamble to the amended Biological Diversity (Amendments) Act 2021.

Tackling cost inefficiencies in monitoring the production and quality assurance process: Most often, seed producers, regulatory authorities, companies encounter crippling production issues and witness cost-inefficiencies in tracking the production process. This is

where using futuristic technologies such as an open-source geographic information system (GIS), a satellite-based remote sensing system, and drones can help in remotely conducting site selection for a specific crop and its quality regulations.

This can be addressed by using an AI-powered business intelligence tool that assists in the data collection and review process. This 'data-driven' tool can offer actionable insights into what's working and, more importantly, what's not so that corrective measures can be implemented with speed and efficiency. The end result is higher seed output at optimized costs and improved quality, leading to increased profits. Transparency is in-built in the process.

As per the claim of Feistritzer (1975) we all witness that seed is the carrier of new technologies – accurately and symbolically. Seed quality is the culmination of the correct application of multiple agriculture inputs and regulations at appropriate stage. Technology is the efficient enabler. Experts claim that the green revolution was, in fact, a form of seed revolution, owing to the production of pure seeds possessing beneficial qualities such as high generation, high physical purity, high vigour, and sound health. Today, rapid advancements in AI-powered technology make this reality easily possible and pave the way for the next revolution. Using technology is becoming the gold standard for seed companies to ensure higher yield, greater transparency, and end-to-end traceability of seeds during production.

By leveraging emerging technologies such as IoT, Big Data, Block chain, etc., seed companies can live up to the promise of high-quality seed production. This advanced technologies facilitate traceability solution is one such tool that promises quality regulation authorities, adulteration-free, high-quality seeds.

What success will look like.

A timely, flexible, and up-to-date seed certification and regulatory system that responds to members and broader agriculture and food sector needs. Continued national and international recognition of the Seed Certification and Regulatory System, Communication and engagement with membership / stakeholders is enhanced, associated contributions and needs are understood and professional competencies are recognized.

The seed sector is diverse, with actors ranging from diversified group of farmers, large life science companies to small and medium sized family businesses and everything in between. The quality regulatory regime should reflect this spectrum of business models and related interests. A national world-class, single window, online accessible system for all seed certification and related regulatory services is need of hour.

Concluding remarks:

There are exists policy and legal frameworks regime in every countries in terms of variety release, plant variety protection, seed production, certification, marketing, import

and export, and protocols harmonization. However, in addition to certain policy gaps, operationalization of the existing legal frameworks is still challenging.

Hence an appropriate amendments in order to address the certain issues indicated in the existing seed legislations like the Seeds Act 1966; Seed Rules 1968;Seeds (Control) Order 1983 and the Environmental (Protection) Act 1986 including exchange of germplasm and the International Treaty on Plant Genetic Resources for Food and Agriculture; Harmonisation of protocols for the facilitation in Seed Trade – Domestic and International certification, seed testing, sanitary and phyto-sanitary measures; Protection of Plant Varieties etc., are the key challenges and strategies shall need to be addressed so as to enable efficient seed quality regime implementation on future perspective note.

An efficient, well-regulated dynamic seed system that meets quality standards, adapts to climate change and market conditions, has transparent and inclusive governance, and maintains biodiversity: a system that provides farmers with certified seed of improved varieties of key crops in sufficient quantity and quality, at a required place and time, with affordable price through multiple production and marketing channels.

Taking into account the evolution of analytical techniques such as molecular biology, machine vision and near infra-red spectroscopy, high resolution imaging techniques to provide more efficient and effective analysis of quality attributes such as GMOs, pathogens and vigour. The rapid growth in the volume of domestic and international trade of seeds has given rise to many challenges, not least of which is the need to harmonize certification procedures and to adopt reliable and enforceable standards. Good cooperation between countries and all stakeholders including international organizations is a response to the need to develop a market-responsive regulatory approach. Every country will continue to be faced with a different legal system and institutional structure and, yet, must compete on the global market.

The OECD Seed Certification system is the most widely used global certification system for the export and import of high-quality seed. The ongoing development and release of new plant varieties and the trend toward the multiplication of seed in third countries increase the complexity of the production and distribution systems. Moreover, increasing cooperation between the public and private sectors is paramount to ensuring that the benefits arising from the use of new varieties are shared between the different shareholders in the system. The adoption of international certification standards has encouraged the growth in the seed trade by reducing technical barriers to trade, increasing transparency, lowering transaction costs and increasing access by farmers in all regions of the world to high-quality seed.

Cooperation and participation in internationally harmonized systems (ITPGRFA, OECD, UPOV and ISTA) is an important means for countries to increase the availability of germplasm, new plant varieties and high quality seed for the benefit of their farmers, without which their ability to respond to the challenges ahead will be substantially

impaired. A predictable, reliable, user friendly and affordable regulatory environment is crucial to ensure that farmers have access to high quality seed at a fair price. Cooperation between international governmental and non-governmental organizations, on the basis of mutual supportiveness, is essential in order to provide effective assistance to governments in the development of an enabling environment. In order to fulfil the important role of the public and the private sector to meet the challenges ahead and hence recognizes the benefits in developing complementarily and synergy between the public and private sectors are equally important. Further urgent government measures and increased public and private investment in the seed sector are required for the long term, if agriculture is to meet the challenge of food security in the context of population growth and climate change.

As seed industry experts and stakeholders we shall need to tackle key topics on science and to further promote innovation, international movement of quality seed, and to take steps to drive engagement with the market access, business practices and value chain. Hence the International seed laws and regulatory frame work in the seed sector needs to take a leadership role in transforming the world's food systems. We've made some important leaps and we need to keep acting to fulfil our vision of a world the best quality seed is accessible to all, supporting food security and sustainable agriculture.

All the regulatory regime discussed in this paper not only affect domestic but also world seed trade between which countries trade occurs, how it takes place and the shape of the agricultural sector within the exporting and importing countries. Despite the existence of a number of regulatory bodies, whose objectives are to harmonize regulations and encourage regional or international seed trade, there are still considerable differences in seed laws and regulations on a national scale. These differences mean that seed cannot always move freely between countries. It also highlights the importance of international schemes that require Member countries to satisfy a number of criteria, thus assuring that necessary standards for safety and quality are met for the movement of seed throughout the world. Predictable and reliable regulatory environments will facilitate trade between trading parties, thereby ensuring that high-quality seed can be made available to farmers.

Acknowledgements and Documents referred:

1. OECD Seed Schemes Brochure 2021 & OECD Seed Schemes Rules and Regulations 2023.
2. ISTA Seed Quality Assurance Documents 2023.
3. Annual Report of ICAR, New Delhi 2022-23.
4. Annual Report of MOA& FW, GOI 2022-23.
5. An overview of national and international seed quality assurance systems and strategies for energizing seed production chain of field crops in India, S Rajendra

Prasad, J S Chauhan and K V Sripathy; Indian Journal of Agricultural Sciences 87 (3): 287–300, March 2017.

6. EU Seed Law Reform and New Genetic Engineering, 5 July 2023, ARCHE NOAH &ARC 2020 Website.

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Seed Sampling: Principles & Procedures

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In a laboratory, only a small portion (quantity) of a seed lot can be examined. Therefore, it is important that this small portion is representative of the seed. Hence, drawing of representative sample correctly is fundamental in order to obtain uniform, accurate and reproducible results. The reliability of the interface made about the quality of the seed lot depends primarily on two components: the accuracy with which the sample represents the lot and the accuracy and precision of the laboratory test. It is observed in many cases that the variations in test results are due to the variation in the sampling. Hence, seed sampling is one of the basic components responsible for the accurate seed testing results. Therefore, utmost care is required for drawing the sample. No matter how accurately the laboratory tests are done, the results can only show the quality of the sample submitted for analysis; consequently the sample should accurately represent the composition of the seed lot.

In his foreword to the ISTA Handbook on Seed Sampling (1986), late Dr Arne Wold, Former Chairman of Bulking and Sampling Committee of ISTA wrote "Sampling of seeds is an important part of seed quality control. Correct sampling is a pre-requisite for the reliable estimation of the quality of a seed lot. Accurate description and detailed information of the sampling procedures are therefore necessary. Uniformity in sampling seed lots as well as drawing working samples is as important as uniformity in test methods in order to obtain accurate and reproducible results, Incorrect sampling may lead to misleading test results, discarding seed lots of high quality or to the approval of seed lots of low quality, which may reduce crop yield or even result in complete failure".

Seed sampling refers to the selection of a small portion of seed from a larger amount. It is aimed at obtaining a sample of a size suitable for tests, in which the probability of a constituent being present is determined only by its level of occurrence in the seed lot.While selecting samples, equal amounts should be drawn from different parts of the seed lot – from the top, middle and bottom of the lot. In general, seed lots are either in bags, in bulk or in stream. The amount or size of a seed lot determines how much sampling is required. This is called the sampling intensity. Seed Testing Laboratory personnel are not necessarily engaged in the sampling of seeds. But, nevertheless they should be well acquainted with the principles of seed sampling and should also be able to guide properly the persons engaged in this job.

Objectives

1. Sampling is done to get a uniform and representative sample from a seed lot, to minimize the errors during seed testing. The size of the submitted sample required for testing is small as compared to the size of the lot. Therefore, care

must be taken to ensure that the submitted sample represents the lot of the seed to be tested.

2. Hence it is essential that the samples be prepared in accordance to ISTA rules to ensure that the small sized sample should represent truly and in the same proportion all constituents of seed lot.

Definitions

Seed lot

A seed lot is a specified quantity of seed that is physically and uniquely identifiable.

Primary sample

A primary sample is a portion taken from the seed lot during one single sampling action.

Composite sample

The composite sample is formed by combining and mixing all the primary samples taken from the seed lot.

Sub-sample

A sub-sample is a portion of a sample obtained by reducing a sample.

Submitted sample

A submitted sample is a sample that is to be submitted to the testing laboratory and may comprise either the whole of the composite sample or a sub-sample thereof. The submitted sample may be divided into sub-samples packed in different material meeting conditions for specific tests (e.g. moisture or health).

Duplicate sample

A duplicate sample is another sample obtained for submission from the same composite sample and marked "Duplicate sample".

Working sample

The working sample is the whole of the submitted sample or a sub-sample thereof, on which one of the quality tests described in these ISTA Rules is made and must be at least the weight prescribed by the ISTA Rules for the particular test.

Sealed

Sealed means that a container in which seed is held and closed in such a way, that it cannot be opened to gain access to the seed and closed again, without either destroying the seal or leaving evidence of tampering. This definition refers to the sealing of seed lots, as well as of seed samples.

Self-sealing containers

The 'valve-pack' bag is a specific type of self-sealing container. It is filled through a sleeve-shaped valve which is automatically closed by the completion of filling the bag.

Marked/labeled

A container of a seed lot can be considered as marked or labeled when there is a unique identification mark on the container, which defines the seed lot to which the container belongs. All containers of a seed lot must be marked with the same unique seed lot

designation (numbers, characters or combination of both). Making of samples and sub-samples must ensure that there is always an unambiguous link between the seed lot and the samples and sub-samples.

General principles of seed sampling:

A composite sample is obtained from the seed lot by taking primary samples from different positions in the whole seed lot and combining them. From this composite sample, sub-samples are obtained by sample reduction procedures at one or more stages forming the submitted sample and finally the working samples for testing. Sampling and sample reduction must be performed using appropriate techniques and equipment that is clean and in good condition.

1. Sampling should be carried out only by persons trained and experienced in seed sampling and employed by the official organizations.
2. The seed lot 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. When there is definite evidence of heterogeneity, sampling should be reduced. In case of doubt, heterogeneity can be tested.
3. The size of the seed lot should also not exceed maximum seed lot size limits as prescribed in the rules, subject to a tolerance of 5%.
4. When sampling is being done by hand, great care should be taken to keep the fingers tightly closed around the seeds so that none may escape. Seed sampler may request that bags be 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 that is, from the lower layers in bags and bins.
5. Other things being equal, a large sample is more representative of a lot than is a small sample. Moreover, if there is a choice as to whether to reduce a sample before sending it to the laboratory, the larger quantity should be submitted.
6. 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.
7. The sampler must sample the minimum requisite number of bags from the seed lot. The sampling intensity must not be less than that prescribed below:

Table 1: Sampling intensity

Weight of individual container in the seed lot	Weight of lot (kg or number of container)	Number of primary samples
>100 kg	Up to 500 kg	At least 5
	501 - 3000 kg	1 for each 300 kg , but not less than 5
	3,001 -20,000 kg	1 for each 500 kg , but not less than 10
	20,001 kg and more	1for each 700 kg, but not less than

		40
Note: • Applicable for containers of more than 100 kg, or from streams of seed entering containers • When sampling a seed lot of up to 15 containers, regardless of their size, the same number of primary samples shall be taken from each container		
15 – 100 kg Inclusive	1 - 4 containers	3 from each container
	5 - 8 containers	2 from each container
	9 - 15 containers	1 from each container
	16 - 30 containers	15 from the seed lot
	31 - 59 containers	20 from the seed lot
	60 or more containers	30 from the seed lot
Note: • For seed pellets, seed granules, seed mats and tapes, small packets and reels, containers of less than 30,000 seed units must be combined to sample units that not exceeding 20,00,000 seeds. The sampling units shall be regarded as containers. • For containers holding less than 15 kg of seed, containers must be combined into sampling units not exceeding 100 kg (20 Containers of 5 kg, 33 containers of 3 kg or 100 containers of 1 kg). The sampling units shall be regarded as containers.		

When sampling a seed lot of upto15 containers, regardless or their size, the same number of primary samples shall be taken from each container.

8. Care must be exercised in reducing composite samples. Careless splitting of the sample cannot be expected to produce two similar portions.
9. Any seed known 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.
10. While taking samples from machine sewed cotton bags, a few stitches at one of the top corners can be loosen broken and then this break can be closed with hand stapling device after the contents of the bag have been sampled or a self adhesive label shall be affixed to ensure proper sealing and to avoid a tampering.
11. The weight of the sample drawn should not be less than the weight of the submitted sample as prescribed in the ISTA rules.
12. Under seed law enforcement programme, only trained and experienced officials are authorized to undertake sampling and he has to give notice to such intention to the person from whom he intends to take sample. Three representative samples should be taken in the prescribed manner, which should be marked and sealed.

- One sample to be delivered to the person from whom it has been taken
- Second to be sent for analysis to the Seed Analyst of the area.
- Third to be retained for any legal proceedings.
- At least two persons should be present and obtain the signature of both the witnesses on form VIII of the Seed Rules.

- Sampler must verify the information provided on the label as per the requirements of the Seed Act.

  Following information should be checked on label

  i. Kind
  ii. Variety
  iii. Lot Number
  iv. Date of Test
  v. Seller's name & address

13. In case of certified lots, sampler should check the following information on seed certification tag:

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

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

Procedures for sampling a seed lot

Preparation of a seed lot and conditions for sampling

At the time of sampling, the seed lot shall be as uniform as practicable. If there is documentary or other evidence of heterogeneity, or the seed lot is found to be obviously heterogeneous, sampling must be refused/ stopped. In cases of doubt, heterogeneity can be determined. Seed may be sampled in containers or while entering the containers. The containers must be fit for purpose, e.g. must not damage the seed, and must be clean to avoid cross contamination. The containers must be labeled or marked before or just after sampling is completed. The seed lot should be so arranged that each individual or part of the lot is conveniently accessible.

Obtaining primary samples

- The primary samples are drawn with the aid of suitable seed triers / or by hand in case of chaffy /non-free flowing seeds
- When defining the number and/or the size of primary samples, the seed sampler needs to ensure (besides meeting the minimum sampling intensity) that the minimum amount of seed required for the requested test(s) is sent to the testing laboratory and enough seed remains available for obtaining duplicate samples, if requested.
- Primary samples of approximately equal size shall be taken from a seed lot, irrespective of where in the lot or container the primary sample is taken.
- When the seed lot is in bags/ containers, the containers to be sampled shall be selected at random or according to a systematic plan throughout the seed lot.

Primary samples shall be drawn from the top, middle and bottom of containers, but not necessarily from more than one position in any container, unless so specified.

- Closed paper bags may also be sampled in this manner. However, the holes in the paper bags should be closed with self-adhesive tape, duly signed by the sampler
- When the seed is in bulk or in large containers, the primary samples shall be drawn from random positions and depths with the aid of bin sampler.
- In case of chaffy seeds that have not been rendered free flowing, the primary samples are drawn by hand.
- When seed is to be packed in small or moisture-proof containers, (e.g. tins, or plastic bags), it should be sampled, if possible, either before or during the filling of the containers. When this has not been done, a sufficient number of containers shall be opened or pierced for abstraction of primary samples. The sampled containers shall then be closed or the containers transferred to new containers.
- Seeds are also sampled as it enters the containers, i.e. at the time processed seeds are being put into the containers, this can be done with the help of an automatic device or manually. A uniform quantity of seeds may be taken from the seed stream at specified intervals.
- Sampling seed lots of seed tapes and seed mats should be done by taking packets or pieces of tape or mat.
- The instruments being used must neither damage the seed nor select according to seed size, shape, density, chaffiness or any other quality trait. All sampling apparatus must be clean before use to prevent cross contaminations. Triers must be long enough so that the opening at the tip reaches at least half of the diameter of the container. When the container is not accessible from opposite sides, the trier must be long enough to reach the opposite side.

Sampling of seed lots may be done by one of the methods listed below.

a. **Automatic sampling from a seed stream**: Seed may be sampled by automatic sampling devices, provided that the instrument uniformly samples the cross section of the seed stream and the material entering the instrument does not bounce out again. It may be operated either under manual or automatic control. However, the intervals between taking primary samples should be constant.
b. **Manual sampling from a seed stream**: Seed streams may also be sampled by using manual instruments when fulfilling the requirements listed under a sampling stick.
c. **Sampling stick**: Sampling stick (e.g. stick trier, sleeve type trier, spiral trier) consists of two parts, one of which fits loosely inside the other, but tightly enough so that seed or impurities do not slip between them. The outer part has a solid pointed end. Both parts have slots in their walls so that the cavity of the inner part can be opened and closed by moving the two parts against each other by either a twisting or a push-pull motion. The sampling stick may be used horizontally, diagonally or vertically. The spiral trier has slots in a spiral arrangement for their subsequent opening from the tip to handle and may only be used of a size smaller than *Triticum aestivum*. The sampling stick is inserted in the closed position into the container, gently pushing it so that the point reaches the required position. Further, the sampling stick is opened and slightly agitated to allow it to fill completely, followed

by closing gently, withdrawing and emptying the primary sample into a container. Care should be exercised inclosing the sampling stick so that seeds are not damaged.

d. **Nobbe trier**: The Nobbe trier (dynamic spear) is a pointed tube with an opening near the pointed end; seed passes through the tube and is collected in a container. The minimum internal diameter of the Nobbe trier should be wide enough to allow the smooth and free flow of seed and contaminants through the trier. It is inserted at an angle of about 30^{o}to the horizontal plane with the opening facing down and pushed until it reaches the required position and revolve it through 180°. Later, it is withdrawn with decreasing speed from the container, gently agitating the trier to help maintain an even flow of seed, and collect the seed sample coming in a suitable container.
e. **Cargo sampler**: The cargo sampler (bulk sampler) consists of a special type of chamber that is fixed to a shaft. The lower part of the chamber is cone-shaped with a pointed end. To reach a greater depth, the shaft may be lengthened by screwing on successive extensions. There is a closing system in the chamber that may be a collar on the outside of the instrument, a wing connected to a door or a valve with a spring. Some cargo samplers can be closed before they are drawn back from the sampling position; others cannot be closed, so that the filled chamber is open during withdrawal.

   For all species, the minimum inside diameter can be about 35 mm and the depth 75 mm. It is inserted in the closed position into the container and gently pushed vertically into the seed so that the point reaches the required position, pull the cargo sampler back about 10 cm or turn it (depending on the closing system), agitate it slightly to allow it to fill completely, gently close if possible and withdraw it and empty the primary sample into a container. Care should be exercised in closing the cargo sampler, so that the seeds are not damaged.
f. **Sampling by hand**: This method can be used for all species and may be the most suitable method for seed that may be damaged by the use of triers, seeds with wings, seeds with low moisture content, seed tapes and seed mats. For hand sampling seed in containers, all positions inside the containers must be accessible. Containers with layers which are not accessible from the regular opening may have to be cut open, sampled and repackaged. Containers may also be partially or completely emptied during the sampling process to gain access to all positions in the containers. For sampling by hand, clean the hand and roll the sleeve up if necessary, insert the open hand into the container to the required position, close and withdraw the hand, taking great care that the fingers remain tightly closed about the seeds so none may escape, and empty the hand into a receiving pan.

Obtaining the composite sample

The primary samples are compared with each other during sampling. If they appear to be uniform, they are combined to form the composite sample, If not, the sampling procedure must be stopped. When primary samples are collected directly into one container, the content of this container may be regarded as the composite sample only if it appears uniform. If not, it must not be used for obtaining a submitted sample.

Obtaining the submitted sample:

The submitted sample of requisite weight or more may be obtained from the composite sample, either by repeated halving or by abstracting and subsequently combining small portions to an appropriate size. Obtaining subsamples such as seed moisture testing must be carried out in such a way that changes in moisture content are minimal. The composite sample can be submitted to the seed testing laboratory if it is of appropriate size for the tests to be conducted, or if it is difficult to mix and reduce the composite sample properly under warehouse conditions.

Obtaining the submitted sample for determination of moisture content:

Obtaining submitted samples of the required size for moisture testing must be carried out in such a way that changes in moisture content are minimal. Samples must be taken in the following way from the composite sample: first, mix the composite sample by either stirring it or by passing it through a mechanical divider and combining preferably once but not more than three times. Then, take a minimum of three subsamples from different positions and combine them to create the submitted sample for moisture testing.

Obtaining duplicate samples:

Duplicate samples, which were requested no later than at the time of sampling, must be prepared in the same way as the submitted sample.

Dispatch of the submitted sample

- The submitted sample should be sealed and marked with the same identification as the seed lot, in such a way that it establishes connection between the seed lot and the sample. The label should contain all the necessary details; such as variety, class of seed, quantity in the lot , to whom it belongs, name of the producer, seed treatment, date of harvesting and threshing, if known, sampled by, date of sampling and kind of tests required.
- For an ISTA International Seed Lot Certificate, the sample must be sealed. The additional information required as well as the name of any chemical treatment applied must be provided.
- After marking, samples should be packed so as to prevent damage during transit. For germination tests, it should be packed preferably in the cloth bags. Submitted samples for germination tests, viability tests and health tests may only be packed in moisture proof containers if suitable storage conditions can be assured.
- For determination of seed moisture content, it should be packed separately in moisture proof containers from which as much air as possible have been excluded.
- Submitted samples shall be dispatched by the sampler to the seed testing laboratory without delay.

Procedure for obtaining the working sample:

Minimum size of working sample

Minimum sizes of working samples are prescribed in the ISTA rules for each test. The working sample weights for purity analyses are calculated to contain at least 2500 seeds. These weights are recommended for normal use in purity test. The sample weights, for counts of other species are 10 times the weights recommended for purity analysis in column 4, subject to a maximum of 1000g.

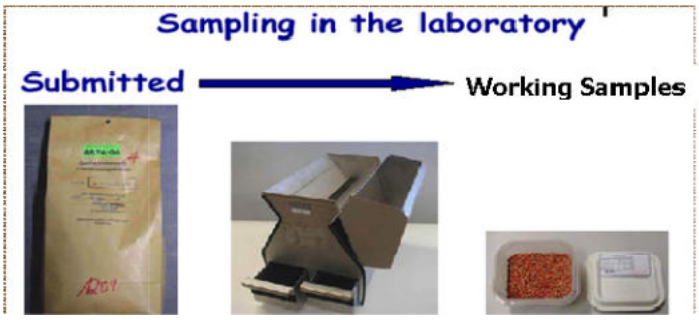
Working sample for all coated seeds except those defined as treated seeds must contain at least the number of seeds, pellets, granules as prescribed in the ISTA rules. If smaller sample is used, the actual number of pellets, seeds or granules in the sample must be reported.

Sample reduction methods

- If the seed sample needs to be reduced to a size equal to or greater than the size prescribed, the seed sample shall first be thoroughly mixed. The submitted/working sample shall then be obtained either by repeated halving or by abstracting and subsequently combining small random portions. One, two or more of these methods may be used in one sample reduction procedure. While using one of the dividers described for seed pellets the distance of fall must not exceed 250 mm.
- Only the spoon methods and the hand halving method may be used in the laboratory to obtain working samples for seed health testing where other samples or equipment may be contaminated by spores or other propagation material.
- For seed tapes and mats, take pieces of tape or mat at random to provide sufficient seeds for the test.
- After obtaining a working sample or half-working sample, the reminder shall be re-mixed before a second working sample or half-working sample in obtained.
- Sub-samples for moisture content determination may be taken in the following way; before taking the sub-sample, mix the sample by either stirring the sample in its container with a spoon or place the opening of the original container against the opening of a similar container and pour the seed back and forth between the two containers. Take a minimum of 3 sub samples with spoon from different positions and combine them to form a sub-sample of the required size. The seed may not be exposed to the air during sample reduction for more than 30 seconds.

Methods for obtaining working samples

- The seed samples received in the Seed Testing Laboratory (Submitted sample) are required to be reduced to obtain working samples for carrying out various tests.

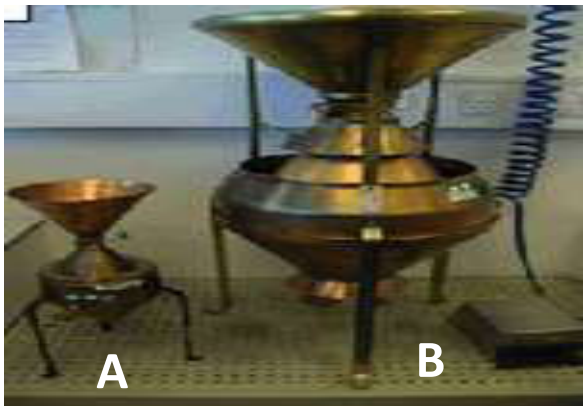


Mechanical divider method

This method is suitable for all kinds of seeds, except some very chaffy seeds. The apparatus divides a sample passed through it into two or more through it into two or more approximately equal parts. The submitted sample can be mixed by passing it through the divider, recombining the parts and passing the whole sample through a second time, and similarly, a third time if necessary. The sample is reduced by passing the seed through repeatedly and removing parts on each occasion. This process of reduction is continued until a working sample of approximately, but not less than, the required size is obtained.

a. Conical divider.

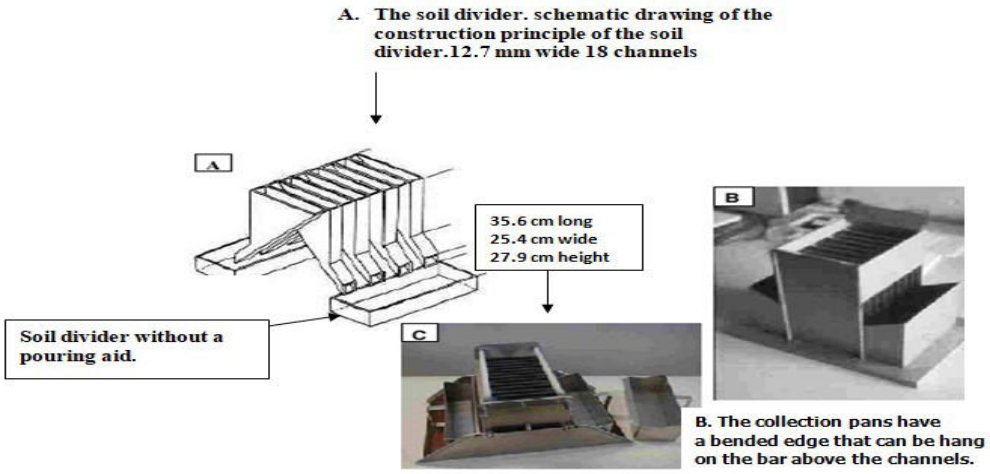
The conical divider (Boerner type) consists of a hopper, cone, and series of baffles directing the seed into two spouts. The baffles form alternate channels and spaces of equal width. They are arranged in a circle and are directed inward and downward, the channels leading to one spout and the spaces to an opposite spout. A valve or gate at the base of the hopper retains the seed. When the valve is opened the seed falls by gravity over the cone where it is evenly distributed to the channels and spaces, then passes through the spouts into the seed pans. Dividers with more than 18 channels have been found to be suitable. Channels must be wide enough to allow the smooth free flow of seed and contaminants. Channels and spaces must be wide enough to low allow the smooth free flow of seed and contaminants. The more channels and spaces, the better the accuracy. Typical conical dividers have about 15 channels and spaces.



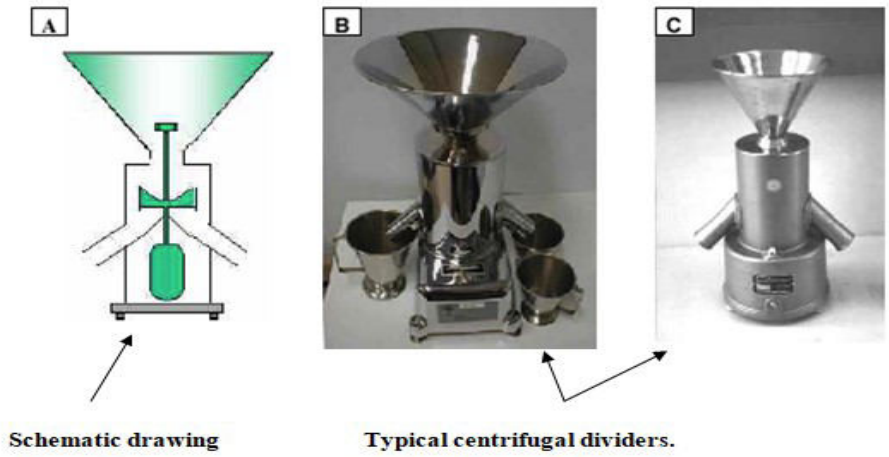
i. **Small divider**: 40.64 cm high and 15.24 cm in diameter, designed for small free-flowing seeds there are 22 channels and 22 spaces, each 7.9 mm wide.

ii. **Large divider**: 81.28 cm high and 36.83 cm in diameter, designed for large seeds and grains, there are 19 channels and 19 spaces, each 25.4 mm wide.

b. **Soil divider (Riffle divider):** It is simpler divider, built on the same principle as the conical divider. However, the channels are here arranged in a straight row instead of a circle as in the conical divider. It consists of a hopper with 18 attached channels or ducts alternately leading to opposite sides, a frame to hold the hopper, two receiving pans and a pouring pan. The channels must be wide enough to allow the smooth free flow of seed and contaminants. The more channels, the better the accuracy. A minimum of 10 channels is required. While using the divider, the seed is placed evenly into a pouring pan and then poured in the hopper at approximately equal rates along the entire length. The seed passes through the channels and is collected in two receiving pans.



c. **Centrifugal divider:** The centrifugal divider (Gamete type) makes use of centrifugal force to mix and scatter the seeds over the dividing surface.The seed flows downward through a hopper onto a shallow rubber cup or spinner. Upon rotation of the spinner by an electric motor, the seeds are thrown out by centrifugal force and fall downward. The circle or area where the seeds fall is equally divided into two parts by a stationary baffle so that approximately half the seeds fall in equally divided into two parts by a stationary baffle so that approximately half the seeds fall in one spout and half in the other spout. The centrifugal divider tends to give variable results, unless the spinner is operated after having poured the seed centrally into the hopper.



Schematic drawing Typical centrifugal dividers.

d. Rotary divider: The rotary divider comprises a rotating crown base unit usually with 6 to 32 attached subsample containers, a vibration chute and a hopper. The seed is poured into the hopper and the rotary divider is switched on so that the crown/ base unit with the containers rotates with approx. 100 rpm and the vibration chute starts to feed the seed into the inlet cylinder of the rotating crown. The longer duration of the dividing operation, the better the accuracy. The feeding rate and therefore the duration of the dividing operation can be adjusted by the distance between the funnel of the hopper and the chute and the vibration intensity of the chute.

There are two principles:

(i) The inlet cylinder feeds the seed centrally onto a distributor within the rotating crown distributing the seed to all containers simultaneously.

(ii) The inlet cylinder feeds the seed de-centrally into the inlets of the containers rotating underneath the inlet cylinder so that the seed stream is subdivided into a lot of subsamples.

For this type of divider, mixing and dividing takes place in one operation.

e. Variable sample divider:

The variable sample divider consists of a pouring hopper and a rotating tube underneath that rotates with about 40 rpm. The tube distributes the seed stream from the pouring hopper onto the inner surface of a further hopper, which is well fitted into a third hopper all being concentric. In the second and the third hopper, there are slots that can be twisted against each other, resulting in wider or narrower slots. The effect is that a smaller percentage will pass through the slots. Either the smaller sample outside the hoppers or the bigger sample inside the hoppers can be used as the required sample. The position of the two hoppers in relation to each other can be adjusted accurately, resulting in pre-determined subsamples sizes.

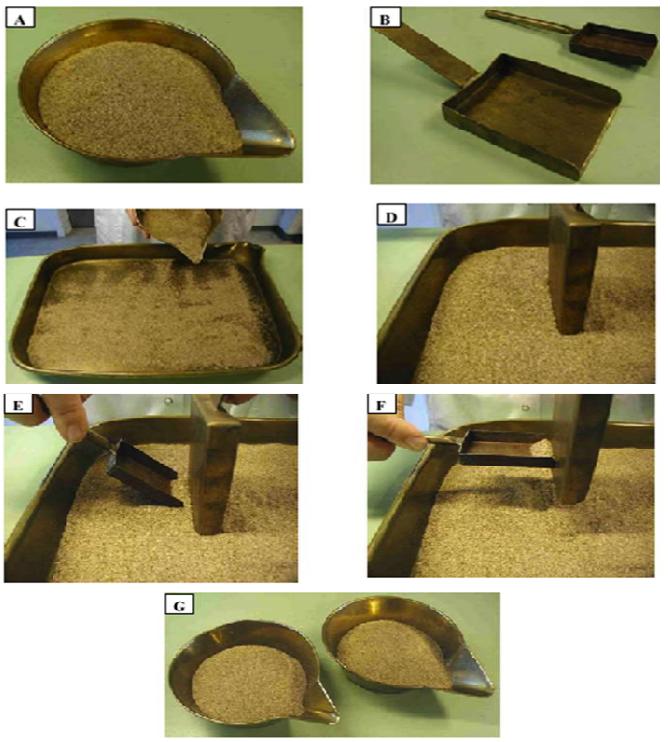
f. Modified halving method:

The apparatus comprises a tray into which fits a grid of equal-sized cubical cells, open at the top and every alternate one having no bottom. After preliminary

mixing, the seed is poured evenly over the grid. When the grid is lifted, approximately half the sample remains on the tray. The submitted sample is successively halved in this way until a working sample, of approximately but not less than the required size is obtained.

g. Spoon method:

- The spoon method is recommended for single small-seeded species and for sample reduction for moisture determination or seed health testing sample reduction for seed health testing.
- For other tests, it is restricted to species with seeds smaller than *Triticum aestivum*; to the genera *Arachis*, *Glycine* and *Phaseolu*s, and to tree genera *Abies*, *Cedrus* and *Pseudotsuga*. For all other species, it can only be used to obtain working samples in the laboratory for seed health tests.
- A tray, a spatula and a spoon with a straight edge are required. After preliminary mixing, pour the seed evenly over the tray; do not shake the tray thereafter.
- With the spoon in one hand, the spatula in the other, and using both, remove small portions of seed from not less than five random places. Sufficient portions of seed are taken to constitute a subsample of the required size.



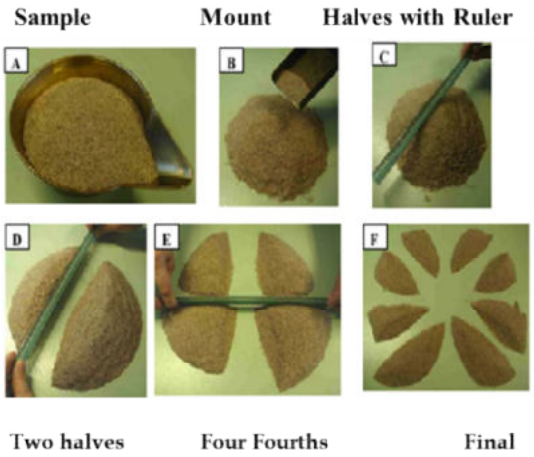
A. Sample to be reduced.
B. Two spoons
C. A spoon is pushed vertically into the seed layer (as a substitute).
D. Distributing the seed over the pan.
E. With the second spoon the seed in front of the vertical spoon is collected.
F. Both spoons are removed from the seed and the seed sample is transferred to a collection pan.
G. Two sub-samples as the result.

h. The hand halving method: This method is the most satisfactory method for chaffy and genera of tree and shrub seed. However, this method is restricted to:

- the following genera of chaffy seeds, *Agrimonia*, *Andropogon*, *Anthoxanthum*, *Arrhenatherum*, *Astrebla, Beckmannioa, Bouteloua, Brachiaria, Briza, Cenchrus, Chloris,*

Dicghanthium, *Chloris*, *Digitaria*, *Echinochloa*, *Ehrharta*, *Elymus*, *Eragrostis*, *Gomphrena*, *Gossypium* (linted seed only), *Melinis*, *Oryza*, *Pennisetum* (non glaucum), *Psathyrostachys*, *Scabiosa*, *Sorghastrum*, *Stylosanthes* (non guianensis), *Trisetum*, *Urochloa*;

- to the following genera of easily damaged fragile seeds: *Arachis, Glycine* and *Phaseolus*;
- and to the following genera and species of tree and shrub seeds: *Acer*, *Aesculus*, *Ailanthus*, *Castanea*, *Cedrela*, *Corylus*, *Fagus*, *Fraxinus*, *Juglans*, *Liriodendron*, *Pinus cembra*, *Pinus pinea*, *Platanus*, *Populus*, *Quercus*, *Salix*, *Tectona*, *Ulmus*.
- This method can also be used with the species, where all other dividing methods are extremely difficult or impossible to use. The steps involved are given below:
- The seed is poured evenly onto a smooth clean surface.
- Thoroughly mix the seed into a mound with a flat-edged spatula
- The mound is divided into half and each half is halved again, giving four portions. Each of the four portions is halved again giving eight portions which should be arranged in two rows of four.
- Combine and retain alternate portions: e.g. combine the first and third portions in the first row with the second and fourth in the second row. Remove the remaining four portions.
- Steps two, three and four are repeated using the retained portions from step four until the weight of sample required is obtained.



i. Random Cups Method

a. Schematic drawing of the tray, the cups and how to distribute the seed over the tray.
b. A tray with cups and distributing a sample over the tray.
c. The tray with the total sample distributed over the tray.
d. The cups removed from the tray and emptied into a glass vessel.
e. Cups of different size in one set.

er is operated

i. Leveled by means of the adjustable feet.
ii. Divider& four containers are checked for cleanliness**.**
iii. A container is placed under each spout.
iv. The whole sample is fed into the hopper; when filling the hopper, seed must always be poured centrally.
v. The spinner is operated and the seed passes into the containers.
vi. Full containers are replaced by empty containers. The contents of the two full containers are fed into the hopper together, the seed being allowed to blend as it flows in. The spinner is operated.
vii. The procedure described in (vi) above is repeated at least once more.

Storage of submitted samples before testing: Every effort must be made to start testing a submitted sample on the day of receipt. Storage of orthodox seeds, when necessary, should be in a cool, well-ventilated room. Non-orthodox (i.e. recalcitrant or intermediated seeds should be tested as soon as possible after obtaining the submitted sample from the composite sample without any storage. Handling of the submitted sample and, if necessary, storage should be done under species specific optimum conditions.

Storage of samples after testing: The primary aim of storage of samples after testing is to be able to repeat the original tests carried out on the submitted sample. Therefore, storage conditions should be such that changes in the seed quality traits tested are minimal. For example, in the case of the purity test or other seed count, the sample should be stored in such a way that the physical identity is kept. In the case of germination, viability or health test of orthodox seeds the sample should be stored under cool and dry conditions. For such tests in recalcitrant and intermediate seeds of tropical and sub-tropical species, long term storage is not possible. For such seed of temperate species storability depends on the fungal status and to some extent whether the seed is dormant or not. All factors pertaining to storage need to be determined on a species basis. Protection against insects and rodents may be necessary. When a re-test in a different testing laboratory is required, a portion shall be drawn from the stored sample and submitted to the designated testing laboratory. The remainder shall be retained in the store.

Table 2: Sample weights of important field and vegetable crops

(1) Crop	(2) Minimum weight of seed lot (kg)	Minimum weight for		
		(3) Submitted sample (gm)	(4) Working sample for purity analysis(gm)	(5) Working sample for count of other species seeds (gm)
FIELD CROPS				
Cereal and Millet crops				
Paddy	20,000	400	40	400
Wheat, Barley and	20,000	1000	120	1000

Triticale				
Oats	20,000	1000	100	1000
Maize	40,000	1000	900	1000
Sorghum	10,000	900	90	900
Pearl millet (Bajra) and Common millet (Proso millet, Hog millet)	10,000	150	15	150
Italian millet	10,000	90	9	90
Barn yard millet and Kodo millet	10,000	80	8	80
Finger millet	10,000	60	6	60
Little millet	10,000	70	7	70
Pulse crops				
Chickpea (Gram)	20,000	1000	1000	1000
Pigeon pea (Red gram, Arhar)	20,000	500	200	200
Green gram (Mung bean)	20,000	1000	120	1000
Black gram and French bean	20,000	1000	700	1000
Lima bean	20,000	1000	1000	1000
Lablab bean, Field bean, Indian bean (Sem) and Horse gram (kulthi)	20,000	1000	500	1000
Cowpea	20,000	1000	400	1000
Garden pea	20,000	1000	900	1000
Lentil	10,000	600	60	600
Chickling vetch	20,000	1000	450	1000
Kidney bean (Moth bean)	20,000	750	75	750
Oilseed crops				
Castorand Groundnut (pods)	20,000	1000	1000	1000
Groundnut (kernels)	20,000	1000	600	1000
Linseed	10,000	300	30	300
Niger	10,000	150	15	150
Rapeseed and mustard	10,000	160	16	160
Rocket salad (Tara mira)	10,000	40	4	40
Safflower	10,000	1000	180	1000
Sesame	10,000	70	7	70

Soybean	20,000	1000	500	1000
Sunflower (hybrids)	20,000	250	125	250
Sunflower (varieties)	20,000	1000	250	1000
Fiber crops				
Cotton varieties (linted)	20,000	1000	350	1000
Cotton hybrids (linted)	20,000	250	25	250
Cotton varieties/ hybrid(delinted)	20,000	350	35	350
Jute (Patsan)	10,000	100	10	100
Roselle (Mesta)	10,000	700	70	700
Sunnhemp	10,000	700	100	700
Forage crops				
Birdwood grass (Dhaman)	20,000	25	3	25
Blue panic, Guinea grass, Setaria grass (Nandi grass) and Shaftal	10,000	25	2	20
Buffle grass	10,000	25	3	25
Cluster bean	20,000	1000	100	1000
Dharaf grass	10,000	-	-	-
Doob	10,000	25	1	10
Egyptian clover (Berseem) and Fenugreek (Methi)	10,000	60	6	60
Indian clover (Sweet clover, Senji)	10,000	40	4	40
Lucerne	10,000	50	5	50
Marvel grass and Para grass	10,000	30	3	30
Napier grass	10,000	150	15	150
Oats	20,000	1000	120	1000
Rice bean (Red bean)	10,000	-	-	-
Stylo	10,000	70	7	70
Sudan grass	10,000	250	25	250
Teosinte	20,000	1000	900	1000
Velvet bean	20,000	500	50	500
Venezuela grass	10,000	25	5	25
Alfalfa (Lucerne)	10,000	50	5	50
Guinea grass	10,000	25	2	20
Green Manure and Miscellaneous Crops				

Dhaincha	20,000	900	90	900
Hemp	10,000	600	60	600
Indigo	20,000	600	60	600
Poppy	10,000	25	1	10
Sugar beet	20,000	500	50	500
Tobacco	10,000	25	0.5	5
VEGETABLE CROPS				
Bulb crops				
Garlic	40,000	-	-	-
Multiplier onion	40,000	250 bulbs	25 bulbs	250 bulbs
Onion	10,000	80	80	80
Cruciferous vegetables (Cole crops)				
Brussels sprouts, Cabbage, Cauliflower, Broccoli, Karamsag, Knol-kohl, Kohlrabi and Sprouting broccoli	10,000	100	10	100
Chinese cabbage	10,000	40	4	40
Cucurbitaceous vegetables				
Ash gourd, Bottle gourd and Winter squash,	20,000	700	70	700
Bitter gourd	20,000	1000	450	1000
Chow-chow	40,000	250	25	250
Ridge gourd	20,0000	1000	400	1000
Round gourd (Indian squash),Snake gourd, Sponge gourd and Water melon	20,000	1000	250	1000
Cucumber, Long-melon, Musk melon and Snap melon	10,000	150	70	150
Little gourd	10,000	250	25	250
Pumpkin (Kashiphal)	10,000	350	180	350
Pointed gourd	10,000	700	70	700
Summer squash	20,000	1000	700	1000
Green/ Leafy vegetables				
Amaranthus	10,000	70	7	70
Asparagus bean (vegetable cowpea)	20,000	1000	100	1000
Celery	10,000	25	1	10
Coriander	10,000	400	40	400

Fenugreek (Methi) and Parsley	10,000	40	4	40
Lettuce	10,000	30	3	30
Parsnip	10,000	100	10	100
Spinach	10,000	250	25	250
Spinach Beet	20,000	500	50	500
Leguminous vegetables				
Broad bean	20,000	1000	1000	1000
Cluster bean, Dolichos Bean, Field bean, sword bean Velvet bean	20,000	500	50	500
French bean	20,000	1000	700	1000
Goa bean, Jack bean	20,000	500	50	500
Garden pea	20,000	1000	900	1000
Lima bean and Scarlet runner bean	20,000	1000	1000	1000
Root crops				
Carrot	10,000	30	3	30
Celeriac	10,000	-	-	-
Garden Beet	20,000	500	50	500
Garden rhubarb	10,000	450	45	450
Globe artichoke	20,000	1000	120	1000
Jerusalem artichoke	20,000	1000	200	1000
Radish	10,000	300	30	300
Rat tail radish	10,000	300	30	300
Sweet potato and Tapioca	40,000	250 roots	25 roots	250 roots
Turnip	10,000	70	7	70
Fruit crops				
Tomato (varieties)	10,000	70	7	70
Tomato (hybrids)	10,000	7	7	7
Brinjal, Sweet pepper Chillies (Hot Pepper),	10,000	150	15	150
Okra (Bhindi)	20,000	1000	140	10 00
Rat Tail Radish (Mungra)	10,000	300	30	300

References:

- Agrawal P.K. (1993). Handbook of Seed Testing, Dept. of Agriculture and Cooperation, Ministry of Agriculture, Govt. of India, New Delhi, pp: 340.
- International Seed Testing Association (ISTA) (2022). International Rules for Seed Testing. Bassersdrof, CH. Switzerland.
- https://seednet.gov.in/
- https://www.seedtest.org/en/home.html

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Seed Health Testing: An Overview

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ABSTRACT

Seed, the small embryonic plant, is an effective means to introduce plant infections into a new location and provide them with a method of survival from one cropping season to the next. In contemporary agricultural research, a healthy seed lot is essential for a desirable plant population and a successful harvest. Plant pathogens, the majority of which include fungi that spread through seeds are one of the major biotic barriers to seed production in the world. An essential step in managing crop diseases is seed health testing, which looks for infections that are transmitted through seeds. A measure of a seed's health is how free it is from infections. The three main organisations that produce standardised seed health test techniques are International Seed Testing Association (ISTA), International Seed Health Initiative (ISHI), and National Seed Health Systems (NSHS). Reliability, cost-effectiveness, simplicity, speed, sensitivity, and specificity are the primary criteria for choosing test procedures for seed health. PCR is very useful for detecting seedborne diseases because of its many advantageous features. It is essential to examine seeds before utilising them as planting material because seeds allow plant infections to spread and survive. The first line of defence in combating plant diseases spread by seeds is seed health testing and detection.

Keywords: Seed health, Seed infection, Detection, Seedborne plant pathogens, Fungi

INTRODUCTION

A small embryonic plant protected by a layer called the seed coat is known as a seed. Cambridge Dictionary defines a seed as "a small, round or oval object produced by a plant and from which, when it is planted, a new plant can grow." It is the result of the mature ovule, which develops in gymnosperm and angiosperm plants following fertilisation and some internal growth. The fundamental unit of production for all food crops worldwide is the seed. In recent times, seeds have evolved into a global commodity that is traded for germplasm. However, seed is also a highly effective means for plant infections to spread to new areas and ensures their survival from one cropping season to the next. Thus, most nations regularly conduct seed health testing for plant quarantine, quality evaluation, and domestic seed certification (FAO, 2010). For all seed companies, testing for seed health is essential to managing the risk of disease (ISF, 2010).

The health of seed refers primarily to the presence or absence of disease-causing organisms, such as fungi, bacteria and viruses, and animal pests, including nematodes and insects, but physiological conditions such as trace element deficiency may also be involved (ISTA, 2024). In contemporary agricultural research, seed health is a well-recognized

element for the desired plant population and optimal yield (Rahman et al., 2008). Seedborne infections provide a persistent threat and may also be blamed for the spread of plant diseases into previously uninfected areas as well as their resurgence (Gitaitis and Walcott, 2007). Seedborne infections pose a significant risk to the establishment of seedlings (Walcott, 2003). More than ever in the global economy of today, plant diseases spread by seed across great distances, natural barriers, and political boundaries. The ability of crops to establish themselves and reach their maximum potential for yield and value is significantly influenced by the quality of the seeds that are sown.

Fungi that spread through seeds are one of the major biotic barriers to seed production in the world. They cause variance in plant shape, pre- and post-emergence death of grains, and vigour in seedlings, which, in turn, reduces germination to some extent. The introduction of new strains or physiologic races of the pathogen together with novel germplasm from other nations, lack of germination, discolouration and shrivelling, development of plant diseases, and toxin synthesis in the infected seed are all possible outcomes of seedborne infections. More than any other kind of pathogen attacking plants, fungi have a severe negative economic impact on agricultural production because they are the largest group of pathogens being able to cause diseases in almost all the farmed crops and lead to significant yield losses.

Standardized seed health test protocols are published by three main organisations, viz. International Seed Testing Association (ISTA), International Seed Health Initiative (ISHI), and in the United States, the National Seed Health System (NSHS) for use in cross-border trade. Sensitivity and selectivity are two crucial ideas in seed health testing that are closely related to one another. For instance, enhancing the semi-selective media's selectivity may result in a reduction in the target organism's recovery efficiency for all or certain strains. On the other hand, improving selectivity could result in fewer non-target organisms interfering with the assay by acting as competitors or inhibitors, which would raise the sensitivity of detection. Because standard growth media are more complicated and frequently turn toxic, which maybe a result of the build-up of peroxides or other secondary metabolites, semi-selective media may have a greater mean plating efficiency than standard growth media. The rate of seed infection is contingent upon various environmental factors, including elevated relative humidity, optimal temperature, and a high seed moisture content. The investigation of pathogens that infect seeds is essential for assessing the health of the seeds and enhancing their ability to germinate, both of which enhance agricultural yield. An essential first step in the treatment of agricultural diseases is seed health testing to identify seed-borne infections.

SEED HEALTH TESTING:

Seed health testing can be used to confirm whether or not there are seedborne infections (Agrawal, 1995). The word "seed health" refers to the presence of bacteria, viruses, fungi, and animal pests like nematodes and insects in the seed lot. The test that is

employed depends on the organism being examined as well as the test's goals, such as quality control or phytosanitary requirements for exporting seed. It involves looking for pathogens visually on the outside or inside of seeds, checking them macro- or microscopically, and incubating the seeds on agar or damp blotter sheets to find the pathogens under a microscope. For many seedborne infections, there are numerous detection assays available; however, only a small number meet the minimal standards for sufficient seed testing. Seed tests should ideally be straightforward to use and interpret, sensitive, specific, fast, robust, and affordable (Walcott, 2003).

Objectives of Seed Health Testing (ISTA, 2024):

The object of a seed health test is to determine the health status of a seed sample and byinference that of the seed lot. Testing for the health of seeds is vital for four reasons:

1. Seed-borne inoculum may give rise to progressive disease development in the field and reduce the commercial value of the crop.
2. Diseases may spread to previously uninfected areas via imported seed batches.As a result, tests to satisfy quarantine regulations can be required.
3. In order to complement germination testing, seed health testing may clarify seedling evaluation and the reasons behind subpar germination or field establishment.
4. The findings of a seed health test may or may not suggest that a seed lot treatment is required in order to get rid of seed-borne pathogens or lower the risk of disease transmission.

There are several reasons why seed testing is essential:

1. to assess the seed's quality using several criteria related to seed quality;
2. to offer a foundation for pricing and customer distinctions between seed lots and seed suppliers;
3. to identify a seed problem's origin in order to facilitate any necessary repair action; and
4. to permit the transport of seeds across international borders and to satisfy the legal and regulatory criteria for certified seed classes (FAO, 2010).

Amare(2007/8) has listedsix main requirements for the selection of seedhealthtestmethods.Theseare:

1. **Specificity:** the capacity to identify the intended pathogen from any organism that might be present on seeds from a field or retail outlet, hence preventing false positive results.
2. **Sensitivity:**the capacity to identify target organisms, which at low incidence in seed stocks have the potential to be significant in field crops.
3. **Speed:**A slight accuracy compromise could be required in some circumstances to guarantee quick results, but these findings should be followed up with more conclusive testing.

4. **Simplicity:**Reducing the number of stages in the technique can help lessen error margins and allow testing to be conducted by workers who may not be highly qualified.
5. **Cost-effectiveness:**Test expenses ought to be included in the allowable production margins for every crop.
6. **Reliability:**Test procedures need to be sufficiently reliable so that, no matter who conducts the test, results may be repeated both within and between samples of the same stock (within the limits of statistical probability and sample variation).

Based on the general methods used to observe the target pathogen, seed health tests have historically been divided into four separate classes. such as direct inspection, incubation tests, embryo examination (embryo count method), molecular methods and immunoassays.

1.1.1. Directexamination(inspection):

A qualitative and semi-quantitative way of checking the health of seeds is direct examination or inspection of dried seed, wherein the effects of fungal infections on the seed's physical appearance are seen, or the fruiting structures of fungi are identified under a stereomicroscope Sclerotia, smut balls, fungal spores, and other fructifications like pycnidia and perithecia can all be found with this technique.Seeds may become discoloured or smaller if they are heavily affected by certain pathogens.For instance, sorghum seeds infected with acremonium wilt are totally malformed, whereas maize seeds infected with Nigrospora show white streaks with black spore masses near the terminals.(AgarwalandSinclair, 1997).

1.1.2. Incubationtests

To enable pathogens on the seed to proliferate, the seeds are incubated for a predetermined amount of time in the agar plate or blotter test under predetermined environmental conditions.Features including the size, septation, and chain development of conidia, as well as the shape, length, and arrangement of conidiophores, are used to distinguish between different fungi (Warham et al., 1990).

1.1.3. Blottertests/Seedlingsymptomtests

Undoubtedly, one of the most crucial techniques for detecting fungal infections in seeds is the blotter test (Limonard, 1966).Similar to germination experiments, blotter tests include placing seeds on moistened blotter paper layers and incubating them in an environment that encourages fungal development.After allowing the seed to germinate, any relevant indications or symptoms associated with fungal seed-borne diseases may appear. During incubation, the environment has an impact on the pathogen's symptoms. The blotter test indicates whether the seed is infected based on the presence of mycelium and fruiting bodies; in certain cases, it also indicates whether the seedlings are infected based on symptoms on the young plants.Certain experiments include incubating seeds so they can germinate and show symptoms (dark spots on the cotyledons of bean seeds infected by the anthracnose pathogen are an example).In alternative experiments, the purposeful suppression of seed germination facilitates the development of seed-borne infections (For example, the herbicide 2,4-D is sprayed prior to incubation to allow the

pycnidia of seedborne Phoma lingam to develop on Brassica seeds, enabling a larger number of seeds to be analysed)(Limonard, 1966).

1.1.4. Agarplate

Using an agar plate is the most used technique for identifying seed borne fungus.Even in the early stages of the fungus's development, viable fungal material can be found using incubation techniques.To promote the growth of seedborne fungus, seeds are typically placed onto sterile agar media (potato dextrose or malt agars are the most popular types). Agar plate method may be used for toquantitative determination of the fungal load of seed (dilution plate methods) or for qualitativedetermination of the species composition (direct plate method). The dilution plate method is the most widely used technique to quantify the amount of fungal tissue present in kernels.This approach has two variations: the spread plate method, which is preferable at low sample contamination, and the pour plate method, which is used more commonly.However, one of the best ways to ascertain the genus and species composition of the grain fungus is through the use of the direct plating method.Using this technique, entire kernels are positioned on the culture medium's surface after being thoroughly cleaned on the outside.It is advised that the direct plating technique be used to assess the quality of bulk grain, as it is a highly valuable tool for identifying the extent to which fungus have colonised kernels inside.The application of the agar test varies widely, mostly in terms of sample preparation, media selection, incubation temperature, and length of time.Bacterial contamination can be reduced by using acidic agar media (Trojanowska, 1991).On agar, bacterial colonies can occasionally form and impede fungal growth, making identification challenging. This can be resolved by cooling the autoclaved agar medium to 50–55°C before adding an antibiotic, such as streptomycin.

1.1.5. Examinationoftheembryo(Embryocountmethod)

When dry seeds are examined both visually and at 10–30x magnifications, a variety of plant diseases can be seen that either coexist with the seeds as fungus bodies (like sclerotic seeds) or have transformed the seeds into fungus structures (e.g., ergots). Sclerotia of the fungi including Sclerotinia and Typhulaspp. may be mixed with seeds ofcrucifers, clovers, grasses, and seeds of other crops. Grass ergot is caused by the fungus Claviceps Purpurea, which is frequently combined with seed to form ergots. For seedborne diseases that are not detectable through incubation or direct inspection, staining techniques are employed.Testing for the presence of loose smut (Ustilago segetum var. tritici) mycelium in barley embryos is the usual procedure used in seed health testing.

1.1.6. Immunoassays

An increasingly sophisticated testing method is provided by immunoassays; the most popular ones are immunofluorescence and Enzyme Linked Immunosorbent Assays (ELISA).In ELISA tests, a sample is treated with an antibody against a particular protein (antigen) in the pathogen, and the reaction between the two results in a colour shift that denotes infection.For instance, ELISA can be used to identify viruses such as bean pod mottle and soybean mosaic virus.

1.1.7. Molecular techniques

Using molecular biology techniques to diagnose agricultural problems has drawn more attention lately. One key shortcoming of most assays released to date is that they don't go far enough to be useful in a typical plant-health setting.There are molecular methods based on DNA, the most widely used of which is the polymerase chain reaction (PCR), which amplifies pathogen DNA only.After dividing the DNA into distinct sizes using electrophoresis, staining is done.Comparisons with known samples can be used to determine the frequency of pathogen DNA.

1.2. Techniques for Detecting Seed Health

The goal of detection is to determine whether a certain target organism is present in a sample, with a focus on asymptomatic individuals (Lopez*etal*.,2003).Generally speaking, there are two main types of seed health detection assays: traditional seed detection assays and polymerase chain reaction-based seed detection assays.

1.2.1. Traditional assays for detecting seeds

Examining seeds for plant diseases can be a challenging undertaking.Infected seeds may not show any symptoms, rendering visual identification unfeasible, in contrast to infected vegetative plant parts.Furthermore, there may be few pathogen populations on the seeds and an uneven distribution of infected seeds within the lot.For many seedborne infections, there are numerous detection assays available; however, only a small number meet the minimal standards for sufficient seed testing.Seed tests should ideally be straightforward to use and interpret, sensitive, specific, fast, robust, and affordable.Numerous technologies, such as eye inspection, selective media, seedling grow-out tests, and serological procedures, have been used to construct seed assays.(Walcott,2003).

1.2.1.1. Bioassays

Grow-out is arguably the oldest technique for assessing seed health. Because it depends on the uniqueness of the host-pathogen relationship, this process is very selective.Its sensitivity is less certain because inoculation thresholds can change based on the plant cultivar under test, changes in the surrounding environment, fertility, and other elements.Furthermore, as it's frequently required to physically identify individual lesions among thousands of plants, the capacity of plant inspectors to consistently identify low occurrences of disease is a crucial component.However, grow-outs are commonly utilized and acknowledged as the gold standard for identifying a seed lot's level of infection. A positive result is usually indisputable proof that the bacteria was present, alive, and harmful because grow-outs depend on symptom expression (GitaitisandWalcott,2007).

One common grow-out test that is frequently performed is the detection of the bacterial fruit blotch disease, *Acidovoraxcitrulli*(Aac).For the assay to be trustworthy and effective, ideal environmental conditions for the development of the disease must be met. Cross-contamination from other inoculum sources must also be avoided.Using commercial greenhouse potting mixture or steam-sterilized soil, selecting an appropriate sample size of at least 30,000 seeds or 10% seed lot, and creating ideal conditions for seed germination and seedling emergence are additional factors. Frequent disinfestations of planting trays, greenhouse floors, walls, and benches are also important.Relative humidity should be kept at least 70% throughout the test, and it shouldn't be permitted to drop below 50% for

longer than 12 hours.In an ideal world, grow-out temperatures would be between 30 and 32 degrees Celsius, never dropping below 25 degrees or rising over 38 degrees for longer than 12 hours. In an isolated section of the greenhouse, an Aac-infested seed sample should be included as a check to confirm further that the conditions are conducive for symptom expression. The control seed lot should be infested with an Aac strain that carries an antibiotic resistance marker or a distinct DNA fingerprint in order to mitigate the possibility of cross-contamination from these inoculum sources. Ample spacing between seed lots and the construction of plastic barriers 60–90 cm high can help prevent potential issues related to the bacterium's splash dissemination during irrigation.Effective management techniques are also required to reduce the passive spread of diseases by insects, mites, or other vectors.While secondary spread inside a seed lot is desired because it leads to the establishment of infection foci that increase the likelihood of visual detection, these procedures are important to prevent contamination between seed lots(Gitaitis and Walcott, 2007). Up to the assay's conclusion, cotyledons and true leaves should be inspected every day following germination.Every seedling should be checked for symptoms in well-lit natural light settings.Certain plants frequently experience natural water congestion; this is not to be confused with the water soaking that leads to the development of diseases.Apart from removing sick seedlings for testing and isolation, all contact with seedlings should be avoided until the final inspection.Workers should either sanitize their hands before handling seedlings or wear disposable gloves that can be replaced between seed lots. After three weeks if there are no visible symptoms, a seedling grow-out experiment can be stopped; if symptoms are present, bacteria should be isolated and further diagnostic tests (such as immuno-strips, PCR, and/or pathogenicity) should be performed to determine the pathogen's identity (Gitaitis andWalcott, 2007).

1.2.1.2. Serologicalmethods(Immunoassays)

In serological seed testing, specific antigens on the surfaces of plant diseases are recognized by antibodies which could be either monoclonal or polyclonal. (Hampton *et al*., 1990). When antibodies attach firmly and precisely to their target antigens, fluorescent tags or substrates can be broken down enzymatically to reveal the presence of the antibody. Since serological assays don't require pure isolations of the pathogen, they can be used to diagnose both necrotrophic and biotrophic seedborne infections.(Walcott, 2003). The most popular method for detecting seedborne viruses in the past was serology, which has shown to be reliable and sensitive. (Bossennec and Maury, 1978;Falk and Purcifull, 1983; Delecolle*et al*., 1985; Barba, 1986; Pasquini*et al*., 1998). The commonly used enzyme linked immunosorbent assay (ELISA) (McLaughlin and Chen, 1990) and immunofluorescence microscopy (Franken, 1992) are two methods for serology-based seed testing.Agglutination tests, immunofluorescence microscopy (IF), immunofluorescence colony-staining (IFC), enzyme-linked immunosorbent assays (ELISA), Western blot, lateral flow devices (e.g., immunostrips), flow cytometry, and immunocapture techniques like immunomagnetic separation (IMS) are some of the serological methods used to detect and identify bacterial pathogens(Munkvold, 2009).

The utilization of infrared fluorescent (IF) in Georgia's black rot seed certification scheme was discouraged due to the challenge of determining a threshold of positive fluorescent cells that can result in crop disease development.Researchers and regulatory

staff came to the conclusion that IF produced an excessive number of false positives, possibly as a result of the bacteria attaching to nonviable cells or exposed antigenic determinants (Munkvold, 2009).To address the issue of possible false positives, strategies like IFC were created (Glynn et al., 2008).In the IFC Seed Health Assay, seed extract is combined with an equivalent volume of agar medium.After drying and incubating, the mixture is exposed to antibodies specific to the target bacteria that have been coupled with a fluorescent dye.Fluorescence microscopy can be used to visualize colonies dyed with the antibody-dye conjugate. Glass capillary tubes can be used to collect the bacteria within the colonies and move them to an appropriate growth medium.Assays evaluating the health of seeds have also employed ELISA. Like the IF, ELISA is generally accused of yielding an excessive number of false-positive responses (Munkvold, 2009). Pantoeastewartii ssp. stewartii was effectively identified by ELISA in maize seeds (McCornack and Ragsdale, 2006).For assessing the health of seeds, the double antibody sandwich (DAS)-ELISA method using polyclonal and monoclonal antibodies proved the most suitable.Afterwards, by creating a response curve that connected absorbance values with the quantity of bacterial CFU collected, the technique was utilized to measure populations of P. stewartii ssp. stewartii in individual seeds (ISTA, 2007).

Another interesting serological method for application in seed health assessments is flow cytometery (Broders et al., 2007). It automatically sorts and analyzes bacterial cells that have been tagged with dye-conjugated antibodies while they are in suspension.Thus, by measuring the amount of light scattering and fluorescence emitted by thousands of individual cells retrieved from an infected seed sample, several parameters can be ascertained in a matter of minutes.A laser beam lights the tagged bacterial cells and activates the fluorescent tags on the antibodies as the stream of cells flows through a flow cell.Multiple metrics, including cell size, granularity, and cell roughness, can be assessed simultaneously by utilizing distinct fluorescence detectors.Flow cytometry can also be used to assess the vitality of target cells when combined with fluorescent probes that target membrane potential, respiratory activities, or important enzymes.Using antibody-antigen specificity is also possible with immunomagnetic separation (IMS) PCR.IMS separates target cells from heterogeneous mixtures using tiny magnetic polystyrene beads covered with antibodies (Ojeda and Verdier, 2000).Following their immobilization using a magnet particle concentrator, the immunomagnetic beads are cleaned to get rid of any nontarget bacteria and inhibitory substances.Captured cells can be plated onto a semiselective medium, or template DNA can be extracted by boiling the cells and utilized for PCR. Walcott et al. (2003) demonstrated that IMS-PCR, as opposed to traditional PCR, increased the detection threshold of A. avenae subsp. citrulli by a factor of 100 in water melon seed assaying.The detection of P. ananatis in onion seeds was also accomplished with the help of IMS, which is essentially a concentration step to effectively enhance the amount of target DNA in the PCR sample.By enhancing the effectiveness and consistency of PCR-quality pathogen DNA extraction from seeds and removing seed components that may impede PCR, IMS-PCR can lower false-negative results (Munkvold, 2009).

Table 1. General features of seed detection assays including the time required for completion, sensitivity, ease of application, specificity, and applicability for the detection of fungi, bacteria and viruses (Tsedaley, 2015)

Assay Time of Specificity	Time of required	Sensitivity	Ease of application	Specificity
Visual examination	5–10min	Low	Simple and Inexpensive (requires experience)	Low
Semi selective media	2–14d	Moderate	Simple and inexpensive	Low–moderate
Seedlinggrow-outassay	2–3weeks	Low	Simple, inexpensive and robust	Low
Serology-baseddetection	2–4h	Moderate–high	Simple, moderately expensive and robust	Moderate–high
Conventional DNA extraction and polymerase chain reaction(PCR)	5–6h	High	Complicated; easy to interpret, expensive	Veryhigh
BIO-PCR (selective target colony enrichment followed by PCR)	2-3d	Very high	Complicated, expensive	Very high
IMS-PCR (immune magnetic separation and PCR)	2-5h	Very high	Complicated, expensive	Very high
MCH-PCR(magneticcapturehybridizationandPCR)	2-5h	Veryhigh	Complicated,expensive	Veryhigh
Real-timePCR	40-60min	Veryhigh	Complicated, expensive	Veryhigh
DNAmicroarrays	6 h	Veryhigh	Complicated,expensive	Veryhigh

1.2.2. Detection Techniques Based on Nucleic Acids

The in-vitro, primer-directed enzymatic amplification of nucleic acids is known as polymerase chain reaction or PCR.Numerous various applications, such as the identification of plant diseases, have made use of this technology.In PCR, primers are tiny oligonucleotide probes that are intended to anneal to particular DNA sequences in the chromosomal DNA or RNA of the target organism. These primers then hybridize with the target sequence to drive the amplification of millions of copies of that sequence.Following electrophoresis in agarose gels stained with ethidium bromide, this amplified DNA can be seen (Walcott, 2003).In the vegetable seed sector and in certain official seed testing laboratories for quality control testing, the astonishing expansion of PCR-based

technologies for detecting pathogens in seeds has produced highly helpful tools that are available and have started to be utilized (Agarwal, 2006).Molecular methods, primarily based on PCR, hybridization, or amplification, have been developed for the most significant plant pathogenic bacteria and viruses.The lack of robustness in PCR has hindered its introduction for routine detection, despite the fact that it can achieve excellent sensitivity and specificity (Van der Wolf et al., 2001).PCR is very useful for detecting seedborne diseases because of its many advantageous features. These include speed (completed within 2 to 3 h); specificity (DNA probes can be designed to amplifynucleicacidsfromthedesiredgenus,species,subspecies,race,etc.);sensitivity(singlecopiesofnucleicacidscan be detected after amplification) and easy and objective result interpretation (the presence of a DNA fragment of specific size indicates the presence of the pathogen) (Tsedaley, 2015).Owing to this enormous potential, numerous PCR-based tests for seedborne diseases have been published over the previous ten years.(Pasquini*etal*.,1998).

The use of PCR-based techniques for seed health assessment has been hindered by a number of issues (Walcott, 2003).The financial outlay and technological know-how required to set up PCR capacity can be challenging in developing nations.Poor quality DNA and PCR inhibitors from seed extracts can be technical obstacles that result in misleading negative results, even in cases when price and knowledge are not the primary obstacles.For PCR-based techniques, low sample intensity can also lead to poor sensitivity.The possibility of false positives from the detection of residual DNA from nonviable disease propagules has been a significant barrier to the use of nucleic acid-based seed health testing (Agarwal, 2006).

At first, it was thought that PCR was too sensitive to be used frequently as an assay for seed health.Concerns were raised over PCR's capacity to discriminate between living and dead cells. PCR is a sensitive technique that can potentially detect a single bacterial cell, but it is no more sensitive than many other techniques due to the large sample size and volume of seeds (30,000 seeds/liter of buffer, for example) being tested and the small volume (~4 µl) that can be used as a template in the PCR reaction.Consequently, alternative methods have been employed to identify pathogens in seeds, including nested PCR (Ojeda and Verdier, 2000).Through the use of primers intended to anneal to internal portions of the amplicon generated by the first round of amplification, nested PCR increases sensitivity. *Ralstonia solanacearum*, the causal bacterium of bacterial wilt of tomato seeds was detected using nested PCR by Poussier et al. (2002).The advantages of these quicker procedures must be weighed against the less expensive (but more inconvenient for the grower due to the longer time necessary to identify a disease) isolation-based methods. Nucleic acid-based methods are often more expensive to implement.Currently, quantification is a significant drawback of nucleic acid approaches in seed health testing. The technology is accessible and rather easy to operate. Nonetheless, the expense of the required apparatus continues to be unaffordable for regular seed health examination (Munkvold, 2009).

1.2.2.1. Marker use in seedborne pathogens

This method has also significantly advanced our knowledge of the epidemiology of infections that are transmitted through seeds.Using markers to distinguish seedborne strains from strains originating from other inoculum sources can be crucial for pathosystems involving ubiquitous pathogens or those with multiple infection paths.Naturally occurring genetic markers, such as antibiotic resistance, vegetative compatibility, and molecular markers unrelated to phenotypic, have been the most widely used forms of markers.For many years, antibiotic resistance in bacterial pathogens—whether it be naturally occurring, caused by mutation, or introduced through genetic engineering—has been utilized as an accurate marker. (Munkvold, 2009).

A genetically regulated characteristic known as "vegetative compatibility" characterizes a fungal isolate's capacity to anastomose and create vegetative (asexual) heterokaryons.Members of the same vegetative compatibility group are identified as strains that are compatible with one another (Leslie, 1993).When it comes to fungi with a highly varied vegetative compatibility structure, this characteristic can be utilized to distinguish between endemic and introduced strains.Numerous investigations on the seed transmission of *Fusarium verticillioides* and related *Fusarium* species have employed this methodology.The method frequently uses the pathogen's nitrogen utilization mutants (Klittich and Leslie, 1988) as a tool to identify appropriate reactions.On the other hand, Galperin et al. (2003) found that seedborne inoculum was a substantial source of inoculum for kernel infection of mature sweet corn plants utilizing nitrogen-utilization mutants as marked strains.Using *Stagonosporanodorum* strains distinguished by distinctive AFLP profiles, the role of seedborne inoculum to Stagonospora leaf blotch outbreaks was examined (Bennet et al. 2007).An additional genetic marker for assessing the significance of seedborne *F. verticillioides* is mycotoxin production.By inserting foreign genes into pathogen strains that can function as molecular markers or readily identifiable expression phenotypes, additional markers have been used. According to Munkvold (2009), two instances include genes that express fluorescent proteins and beta-glucuronidase (GUS).

FINAL VERDICT

A small plant in the embryonic stage, a seed, is the fundamental unit of production for all of the world's food crops.It is a highly effective way to introduce plant diseases into a new environment and ensures their survival from one cropping season to the next.In contemporary agricultural research, a healthy seed bank is essential for a desirable plant population and a successful harvest.Fungi that spread through seeds are one of the major biotic barriers to seed production in the world.The three main organizations that produce standardized seed health test techniques are ISTA, ISHI, and NSHS.An essential step in managing crop diseases is seed health testing, which looks for infections that are transmitted through seeds.A measure of a seed's health is how free it is from infections.Reliability, cost-effectiveness, simplicity, speed, sensitivity, and specificity are the primary criteria for choosing test procedures for seed health.PCR is very useful for detecting seedborne diseases because of its many advantageous features.Certain techniques for testing for seed health can be used to identify pathogen locations within

seed tissues, identify the origins of seedborne infections, validate the occurrence of seed transmission and its mechanisms, and determine how external biotic and abiotic factors impact seed transmission or other stages of the disease cycle.Seed must be examined for disease before being used as planting material because seeds allow plant infections to spread and survive. The initial step in treating plant illnesses that are borne from seeds is to test for and detect seed health issues.

BIBLIOGRAPHY:

Agarwal,V.K.2006.*SeedHealth.Int.BookDistributing*.Charbagh,Lucknow,India.554pp.

Agarwa,lV.K.&SinclairJ.B. 1997.Principlesofseedtechnology.2nd Edition.CRS Press,Florida.538pp.Agarwal, V.K. &Graur,A. Undated. Seedhealthtests:seedborne Diseases.

AmareA.,2007/8.DiseaseDiagnosisandTechniquesinPlantPathology.PracticalGuide.Unpublished.Andersen A.M.and.LeachC.M. 2010.TestingSeedsforSeedborneOrganisms

Barba,M.1986.Detectionofapplemosaicandprunusnecroticringspotvirusesinalmondseedsby ELISA.Arch. Phytopathol.Plant Protection22:279–282.

BennettRS,MilgroomMG,SainudiinR,CunferBM,BergstromGC.2007.Relativecontributionofseed-transmittedinoculumtofoliarpopulationsof*Phaeosphaerianodorum*.*Phytopathology*97:584–91

Bossennec,J.M.andY.Maury.1978.UseoftheELISAtechniqueforthedetectionofsoybeanmosaic virusinsoybeanseeds.Ann.Phytopathol.10:263–268.

BrodersKD,LippsPE,PaulPA,DorranceAE.2007.Evaluationof*Fusariumgraminearum*associated withcornand soybeanseed andseedlingdisease inOhio. *PlantDis.*91:1155–60

Delecolle,B.,H.Lot,andM.J.Michel.1985.ApplicationofELISAfordetecingonionyellowdwarfvirus in garlicandshallotseedsandplants.Phytoparasitica13:266–267.

Falk,B.W.andD.E.Purcifull.1983.Developmentandapplicationofanenzymelinkedimmunosorbentassay(ELISA)testtoindexlettuceseedsforlettucemosaicvirusinFlorida.PlantDis.67:413–416.

FAO,2010.SeedsinEmergencies. Atechnicalhandbook.Plantproductionandprotectionpaper202.

Franken,A.A.J.M.1992.Comparisonofimmunofluorescencemicroscopyanddilutionplatingforthedetectionof*Xanthomonascampestris*pv. *campestris*incrucifer seeds.Neth. J.PlantPathol. 98:169–178.

GalperinM,GrafS,KenigsbuchD.2003.Seedtreatmentpreventsverticaltransmissionof*Fusariummoniliforme*,makingasignificantcontributionto diseasecontrol.*Phytoparasitica*31:344–52

GitaitisR.andWalcottR.,2007.TheEpidemiologyandManagementofSeedborneBacterialDiseases.Annu.

Rev.Phytopathol.2007.45:371–97

Glynn NC, Hare MC, Edwards SG. 2008. Fungicide seedtreatment efficacyagainst*Microdochiumnivale*and *M.majus*invitroandinvivo.*Pest Manag.Sci.*64:793–99

Hampton,R.,E.Ball,andS. De Boer. 1990.Serological methods fordetection andidentification of viral andbacterialplantpathogens:Alaboratorymanual.APS Press, St. Paul, Minn.

International Seed Federation (ISF), 2010. Guidelines on Seed Health Testing in the VegetableSeed Industry.

InternationalSeedTestingAssociation(ISTA),2007.*InternationalRulesforSeedTesting*.Bassersdorf, Switzerland:ISTA

InternationalSeedTestingAssociation(ISTA), (2024). *InternationalRulesforSeedTesting*.Bassersdorf, Switzerland:ISTA, Chapter 7: Seed Health Testing. Available online at: www. https://www.seedtest.org/api/rm/7N75E48MT86P832/chapter-7-seed-health-testing-1.pdf.

KlittichCJR,LeslieJF.1988.Nitratereductionmutantsof*Fusariummoniliforme*(*Gibberellafujikuroi*) .*Genetics*118:417–23

LeslieJF.1993.Fungalvegetativecompatibility.*Annu.Rev.Phytopathol.*31:127–50

LimonardT.,1966.Amodifiedblottertestforseedhealth.Governmentseedtestingstation,Wageningen.

LopezM.M.,BertoliniE.,OlmosA.,CarusoP.,GorrisM.T.,LlopP.,PenyalverR.andCambraM.,2003.

McCornack BP, Ragsdale DW. 2006. Efficacy of thiamethoxam to suppress soybean aphid populations inMinnesotasoybean.*Crop Manag*.doi:10.1094/CM-2006-0915-01-RS

McLaughlin,R.J. and T.A. Chen. 1990. ELISA methods for plant pathogenic prokaryotes, p. 197–204. In: R.Hampton, E. Ball, and S. DeBoer (eds.) Serological methods for detection and identification of viral andbacterialplant pathogens.APS Press,St. Paul,Minn.

Munkvold G.P., 2009. Seed Pathology Progress in Academia and Industry. Annu. Rev. Phytopathol. 47:285–311NiazI.andDawarS.,2009.Detectionofseedbornemycoflorainmaize(*Zeamays*L.).Pak.J.Bot.41(1):443-451.

Ojeda S, Verdier V. 2000. Detecting *Xanthomonas axonopodis*pv. *manihotis*in cassava true seeds by nestedpolymerasechainreactionassay. *Can. J. Plant Pathol.*

Pasquini, G., A.M. Simeone, L. Conte, and M. Barba. 1998. Detection of plum pox virus in apricot seeds. ActaVirol. 42:260–263.

Poussier, S., Jean-Jacques Chéron, Couteau, A.Luisetti, J. (2002). Evaluation of procedures for reliable PCR detection of Ralstonia solanacearum in common natural substrates,Journal of Microbiological Methods,51(3): 349-359,ISSN 0167-7012,https://doi.org/10.1016/S0167-7012(02)00111-2.(https://www.sciencedirect.com/science/article/pii/S0167701202001112)

Rahman M.M.E., Ali M.E., Ali M.S., Rahman M.M. and Islam M. N., 2008. Hot Water Thermal Treatment forControllingSeed-BorneMycofloraofMaize.Int. J. Sustain.Crop Prod.3(5):5-9

Trojanowska K., 1991. Evaluation of cereal grain quality using mycological methods. In: Chelkowski(editor),Cerealgrain:Mycotoxins,fungiandqualityindryingandstorage.Elsevier,Amsterdam,pp,185-215.

Tsedaley, B. (2015). Review on seed health tests and detection methods of seedborne diseases. Journal of Biology, Agriculture and Healthcare (ISSN 2224-3208) 5(5). Available online at: https://core.ac.uk/download/pdf/234660796.pdf

Van der Wolf JM, van Beckhoven JRCM, Bonanats PJM, Schoen CD., 2001. New technologies for sensitive andspecific routine detection of plant pathogenic bacteria. In: de Boer SH (ed) Plant pathogenic bacteria.Kluwer, Dordrecht, pp75–77

WalcottRR.2003.Detectionofseedbornepathogens.*HortTechnology*13:40–47

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

WarhamE.J.,ButlerL.D.andSuttonB.C.,1990.Seedtestingofmaizeandwheat:ALaboratoryGuide. CIMMYT. Available online at :https://repository.cimmyt.org/bitstream/handle/10883/576/63511.pdf?sequence=1&isAllowed=y.

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Latest seed testing equipments used in seed testing and their maintenance

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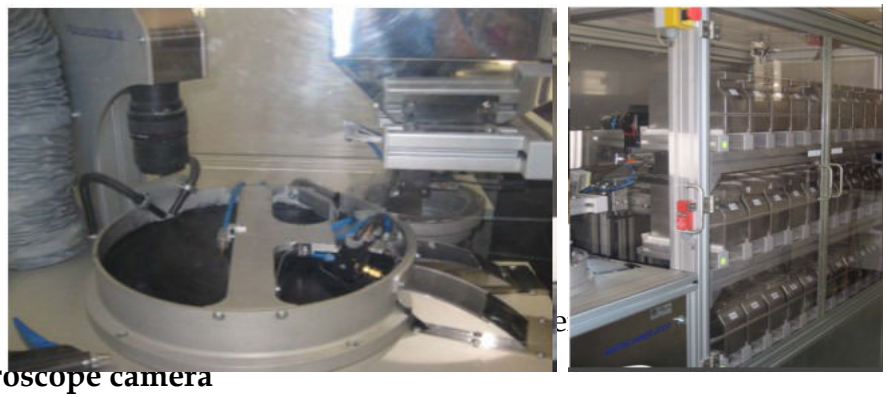
Quality seed availability to farmers is very important to ensure the higher production and productivity of any crops and self sustainably in food production. Seed testing laboratories in India confirm quality of seeds distributed to farmers by conducting various tests. Advancement in seed testing needs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



Digital microscope camera

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

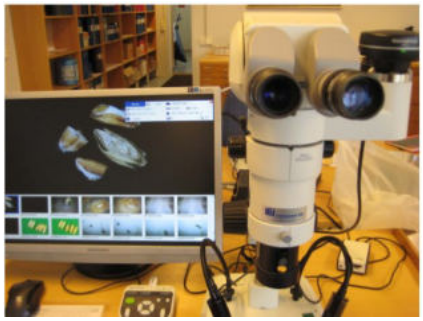


Fig.2 Digital microscope camera

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

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.4 Continuous flowing blower

a. Light weight, immature and diseased seed

b. Medium weight seed

c. Heavy weight selected seed

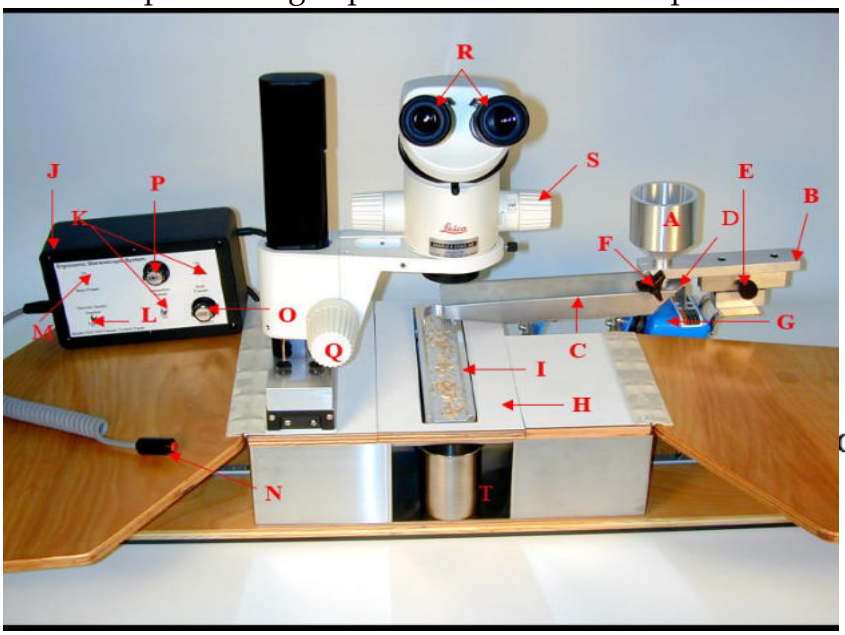
Fig.5. Clover seeds separated by Continuous flowing blower

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).

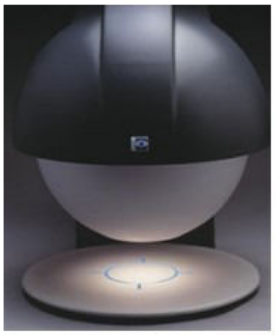


Fig 6. Videometer

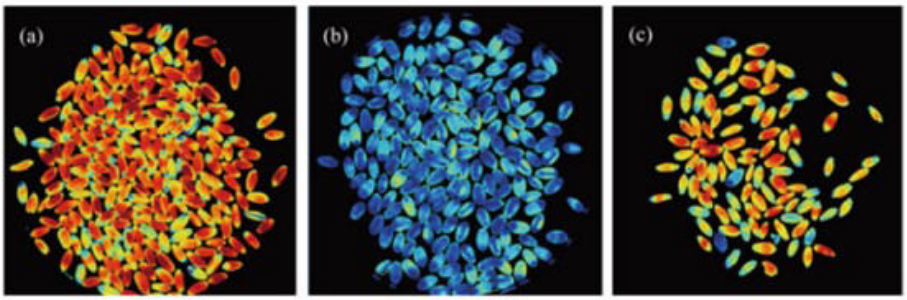


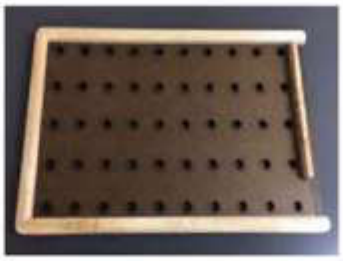
Fig 7. Examples of images generated using the Videometer Lab 2 system (a) 100% T. durum wheat grains; (b) 100% *T. Aestivum* wheat grains; and (c) 10% adultration of *T. durum* wheat grains with *T. aestivum* wheat grains

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).

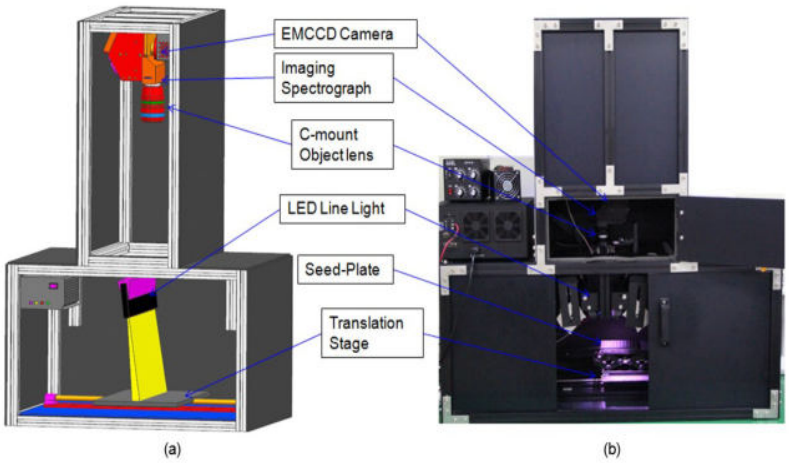


Fig.9. (a) Schematic diagram and (b) photo of the hyperspectral reflectance imaging system

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).

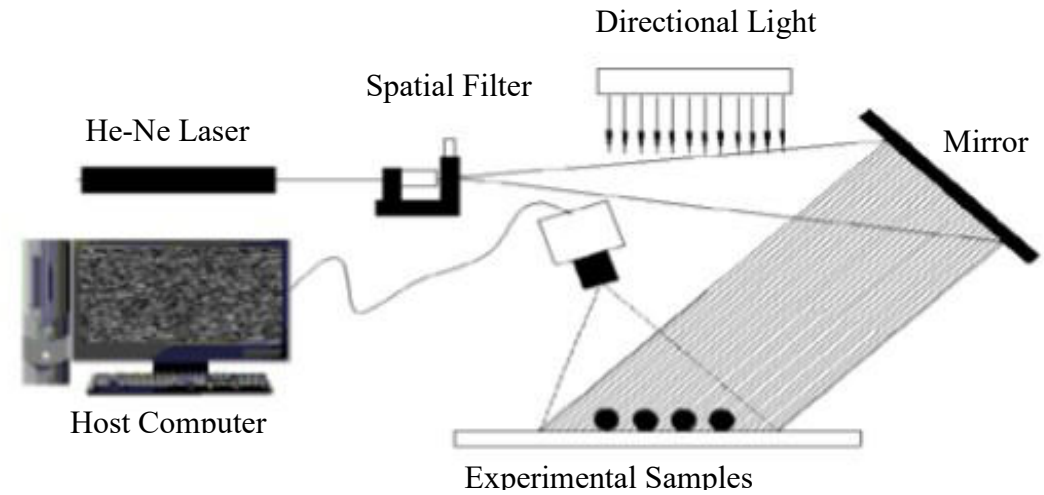


Fig.11. Illumination and image capture experimental system in Dynamic speckle, or biospeckle technique.

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).

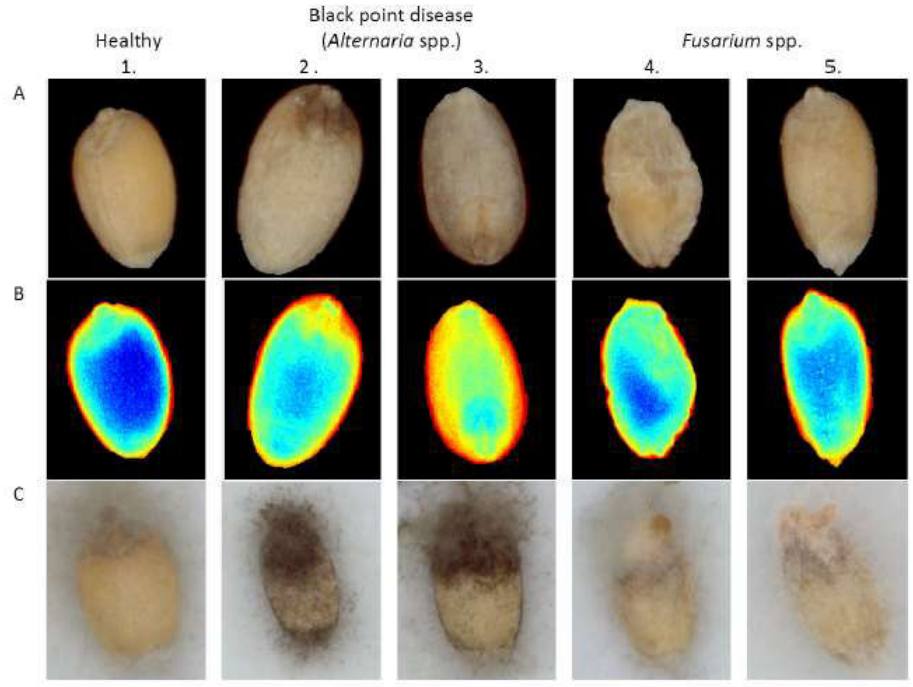


Fig 12. Image comparison of healthy seeds with infected seeds. Seeds on image were infected by black point disease (Alternaria sp.) and Fusarium sp. (A) RGB captured images. (B) nCDA-transformed images. (C) RGB images after seed incubation.

References

Braga R., Rabelo G.F., Granato L.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, Gislum R,Bavec F, Ravn Jørgensen 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

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Modern Techniques for Testing of Transgenic Plants and Biosafety Issues

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Sustaining an ever-growing population has proven to be a formidable challenge, but various advancements and revolutions throughout human history have played a role in bringing this objective within reach. An examination of history reveals that the human population began to increase only after humans began cultivating crops. As a result, they began to transition from a nomadic lifestyle to a settled existence in various regions that were appropriate for crop production, which ultimately led to the formation of civilizations. On the contrary, an abrupt increase in population occurred during the industrial revolution and persisted until the green revolution, as illustrated in the accompanying figure. Man initiated the domestication of untamed species during the agricultural revolution by selecting suitable plant varieties and combining their characteristics via crossing. This domestication has produced commodities in the new world that are easier to process, more productive, and edible than their untamed counterparts. This is supported by phenotypic alterations from the progenitor species to the current form, particularly in crops such as maize, wheat, and rice, the three most significant sources of carbohydrates for humans. The cereals underwent selection with a greater emphasis on production traits (e.g., increased grain yield, increased productive portions per plant) as opposed to survival traits (e.g., seed dispersal efficiency, thick protective seed coats). Throughout the green revolution, which commenced in the mid-20th century, ongoing endeavors were made to select plants that exhibited greater responsiveness to inputs including fertilizers, water, and so forth. Additionally, significant emphasis was placed on developing genetic resistance to both biotic and abiotic factors that posed challenges to crop cultivation. Thus, intensive agriculture gained traction and resulted in the development of genotypes that exhibited enhanced environmental adaptability and resistance to limiting stresses. Numerous novel plant breeding techniques were implemented in conjunction with established methods, including tissue culture-induced somaclonal variations, wide hybridization aided by embryo rescue and other inventive methods to surmount post-fertilization obstacles, induced variability via mutation breeding, and somaclonal variations induced through tissue culture, among others. However,

crossability barriers imposed restrictions on the ability to transfer and combine characteristics from distinct genetic stocks, particularly between unrelated genera. The gene revolution has effectively resolved this challenge by enabling the transfer of desired regulatory sequences and genes into cultivated genotypes, thereby imparting desired traits through a process known as genetic transformation. Thus, modern biotechnology offers a novel instrument for the extremely precise reproduction of plants and animals.

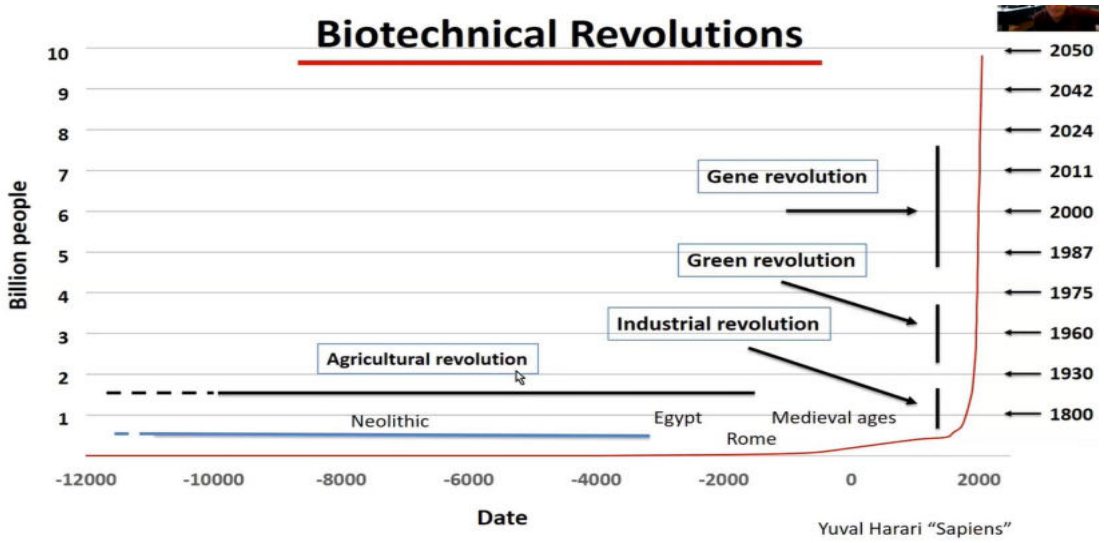


Fig. 1 Different revolutions that have helped in sustaining human population growth

A brief history of transgnenic plants

- In 1983, first genetically engineered (antibiotic resistant) plant tobacco developed
- In 1987, Plant Genetic Systems, founded by Marc Van Montagu and Jeff Schell, genetically engineered insect-resistant (tobacco) plants by incorporating cry genes from *Bacillus thuringiensis (Bt)*.
- In 1994, FlavrSavr tomato produced by silencing the polygalactouronase gene, the first genetically modified crop approved for sale in the U.S.
- In 1995, Monsanto introduced the NewLeaf variety of potato, its first genetically modified crop. Resistant to Colorado potato beetle due to cry genes.
- In1996, 1st genetically modified flower moon dust, bluish coloured carnation, was introduced by inducing the gene for delphinidin production from petunia and transferred to the carnation.
- In 2000, Vitamin A-enriched golden rice, developed by the addition of three beta-carotene biosynthesis genes namely '*psy*' (*phytoene synthase*) from daffodil plant and 'crt-1' gene from the soil bacterium *Erwinia auredorora* and *'lyc' (lycopene cyclase)* gene from wild-type rice endosperm.
- In 2013, Robert Frarley, Mark Van Montagu and Marry Dell Chilton were awarded world food prize for developing first transgenic plant.

Transgenic plants are defined as those in which genetic engineering techniques have been used to modify the DNA of the plants in order to introduce a non-native

trait. Therefore, a transgenic plant is composed of one or more genes that have been inserted artificially via transformation techniques. Once it has been determined that the introduced (engineered) genes are stably integrated, exhibiting the desired phenotype, and conferring it, the plant is deemed transgenic. Therefore, genetically modified (GM) crops exhibit characteristics of specificity and accuracy regarding modifications introduced, provide the flexibility to determine the incorporation of desirable qualities into well-adapted varieties with minimal disruption, and undergo a rapid process in comparison to traditional breeding methods. To sustain an expanding population, produce more desirable products, and generate plants with desired characteristics (e.g., increased yields, crops that endure pests and diseases, and increased shelf life), transgenic plants are required. In order to introduce a new function (gain of function approach), a fully self-contained unit of function (gene along with its own regulatory sequences such as promoter, terminator, and enhancer) could be introduced into transgenic plants; likewise, to eliminate a trait (loss of function approach), post-transcriptional silencing or knockout of the existing gene could be utilized. As a boxed item, a concise history of the transgenic plants is included.through transformation procedures. The plant is considered transgenic only after it is established that the introduced (engineered) gene(s) are stably integrated, expressing and conferring the expected phenotype. Thus genetically modified (GM) crops are specific and precise with respect to changes made, allows flexibility to decide addition of traits into existing well adapted varieties with least disturbance and it is a fast process compared to conventional breeding approaches. Transgenic plants are needed to produce plants with desired traits including increased yields, crops that last longer and withstand pests and diseases, to feed the growing population and to produce more desirable products. Transgenic plants could be produced to introduce a new function (gain of function approach) by introducing a completely self contained unit of function (gene along with its own regulatory sequences such as promoter, terminator and enhancers) or to eliminate a trait (loss of function approach) by silencing post-transcriptionally or by knocking out the existing gene. A brief history of the transgenic plants is provided as box item.

In accordance with the genetic elements employed in the transformation process, four distinct varieties of transgenic plants have been created: transgenics, in which the selected elements and/or gene are foreign to the recipient (e.g., Bt transgenics); cisgenics, in which the donor gene and all regulatory sequences of the transgene are from the same crop species or cross-breedable species as the host; and intragenics, where the donor gene and all regulatory sequences are identical copies of the host's native gene cassette, including their regulatory sequences integrated in the host plant in

the normal-sense orientation; and intragenics, within this category, gene In this scenario, promoters and terminators of distinct genes can regulate gene-coding sequences (with or without introns), so long as the transgene-regulating genes are present in the same pool of cross-breedable genes and genome-edited plants. While the introduced gene constructs are temporarily stored within the plant, the modification they induce is permanent and inherited. Mendelian segregation ensures that introduced gene constructs are eradicated in subsequent generations, leaving the final product genotype devoid of any introduced genes.

Principles of Genetic Engineering: As previously stated, the process of developing transgenic organisms entails the in vitro manipulation of DNA through the utilization of recombinant DNA technology procedures and methods to generate new or novel gene constructs. Therefore, restriction enzymes and ligases are employed in order to generate recombinant DNA molecules from the isolated component sequences (including but not limited to the promoter, terminator, gene, and any cis elements such as enhancers, introns, transcript stabilizers, and so forth). After the recombinant molecule, which is typically a self-contained expression unit, has been synthesized, it is transported to the plant cell of interest to undergo integration, expression, and sustained inheritance.

Steps in gene transformation: Mainly there are four steps

1. Make the transgene construction. This is typically done in *E. coli* though other microbes may be used.
2. Deliver the intended DNA into cells of the recipient organism
3. Select transformed cells using selectable markers such as antibiotic resistance (tetracycline or kanamycin resistance), herbicide resistance (such as glyphosate, glufosinolate), or visual marker genes (*gus/gfp*) or through positive selection.
4. Regenerate whole organism from transformed cells and confirm for the integration, expression and stable transmission of the introduced gene cassette through different molecular methods.

Transfer of recombinant DNA into plant cells: Diverse methodologies exist for the introduction of the engineered gene construct into plant cells. In general, they are categorized as either biological or physical routes. As vehicles, the biological approach will employ alternative organisms, including plant viruses (with the substitution of certain native genes) and Agrobacterium (specifically, Agrobacterium tumefaciens and A. rhizogenes). Physical methods involve the utilization of physical phenomena for the

purpose of delivery, as the name suggests. Several strategies, including microinjection, have been tested and validated; however, gene cannons, biolistics (particle delivery systems, or PDS), and electroporation (typically of protoplasts) are the most prominent examples in this category. Microlaser, Silica Carbide Fibers, and Pollen Tube Pathway.

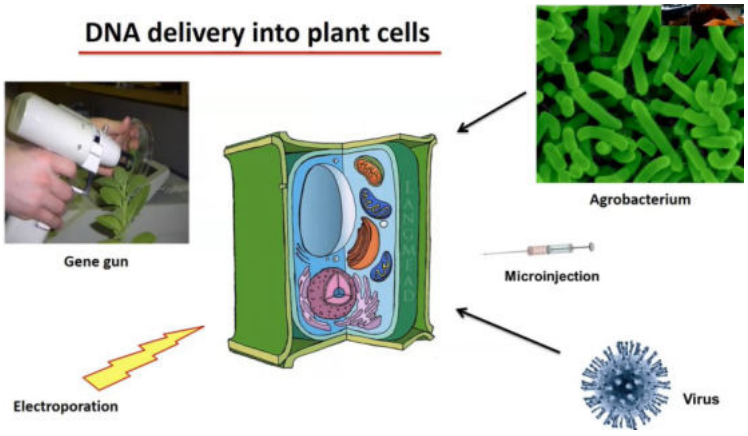


Fig. The main DNA delivery systems into plant cells

Agrobacterium-mediated transformation is widely utilized among the transformation methods and approaches owing to its ability to deliver typically one or two copies of the transgene construct in a clear and precise manner. Agrobacterium, commonly known as crown galls, is a soil-borne pathogen that induces tumor development in a variety of plants. Agrobacterium was found to be the causative agent of this malignancy through the transformation of cells located in the crown region of the plant using a fragment of DNA, commonly referred to as T-DNA for transfer DNA. Left and right border sequences, which are 24–25 base pairs in length and distinguish T-DNA from other DNA, are responsible for limiting the sequence that is conveyed to plants. This T-DNA is a component of the megaplasmid carried by Agrobacterium; it is known as Ti-plasmid (for tumor-inducing plasmid) and it contains genes implicated in the production of opines and plant growth hormones. The continuous division of cells that receive T-DNA at the junction of the transformation process is attributed to the existence of growth hormone-producing genes, ultimately resulting in the development of a tumor. Furthermore, the transformed cells commence opinoid production and secretion; Agrobacterium will utilize these substances as an energy source. As a consequence, Agrobacterium is commonly referred to as "nature's genetic engineer," given that it modifies a plant cell to produce sustenance for itself.

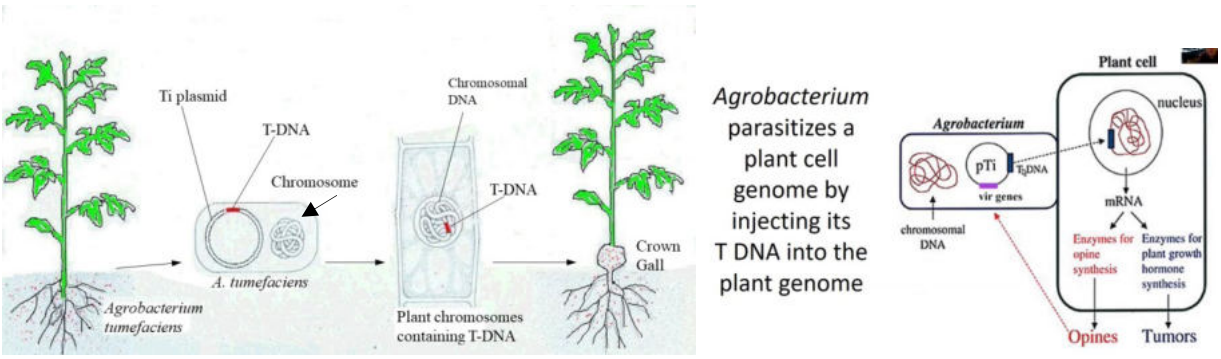


Fig:3. Agrobacterium, a soil bacterium that causes crown gall disease in plants, is a nature's genetic engineer. It processes and transfers a piece of DNA (T-DNA) from its plasmid and tansfoms plant cells into factories that make and export opines which in turn will be utilized by Agrobacterium as food.

By utilizing this inherent capability of Agrobacterium, transgenic plants have been produced. As specified by the breeder, the engineered or modified Agrobacterium will contain the desired gene in addition to selectable markers delimited by border sequences within the T-DNA. Subsequently, this T-DNA will be introduced into the plant. After entering the cell, the T-DNA proceeds to the nucleus, where it is arbitrarily integrated into the genome of the plant cell. It begins to manifest itself upon integration, allowing the transformed cells to withstand selection pressure and consequently become susceptible to selection.

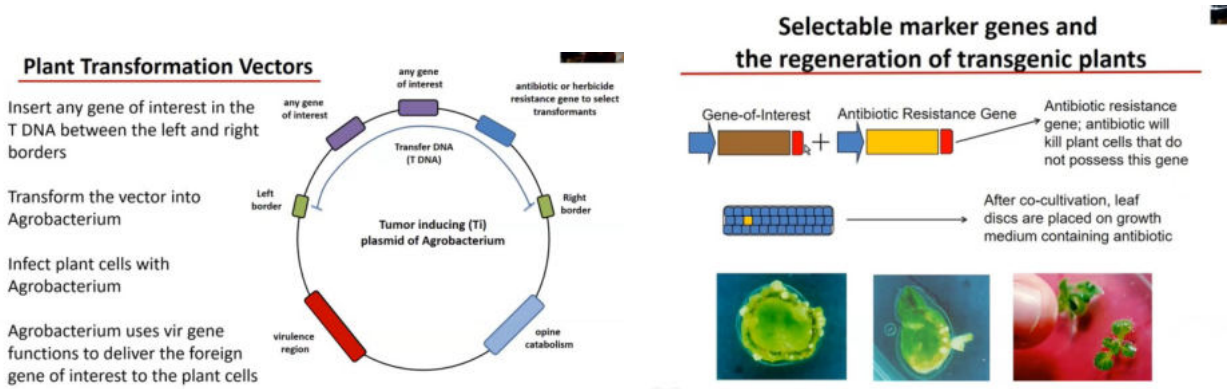


Fig.4 A typical plant transformation vector used in Agrobacterium mediated transformation and how the selectable marker genes are useful in allowing only the transformed cells survice and multiply during the in vitro selection procedure.

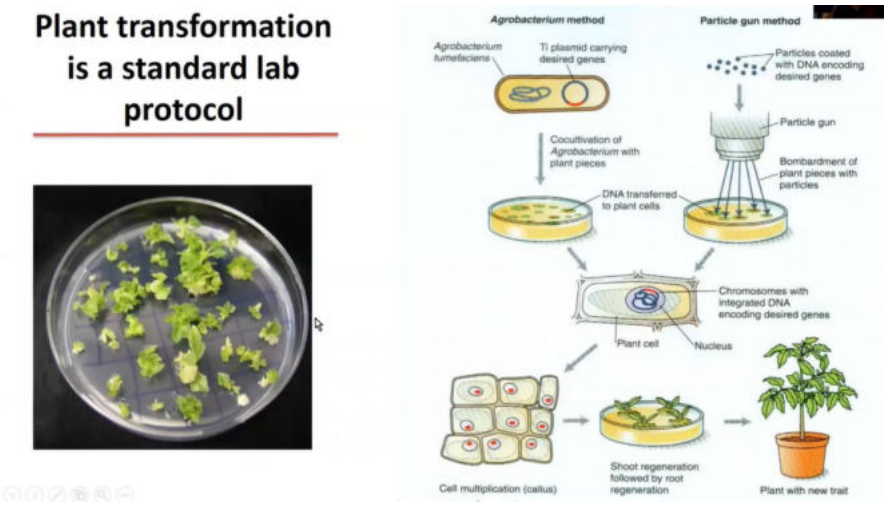


Fig.5 A typical plant transformation protocol followed in the lab

Applications of transgenic plants: Transgenic plants possess a vast array of potential uses, extending beyond the development of commercially viable traits to addressing fundamental inquiries into gene function. The emergence of numerous genomic instruments has led to a deluge of data concerning the function of genes and diverse gene networks in the development of characteristics. In order to determine the functional significance of the chosen genes, one may employ either a gain-of-function or loss-of-function strategy. This shall be accomplished through the generation of transgenics in which the gene function is nullified or by expressing the genes in a genotype devoid of it. Therefore, the development of transgenics has evolved into an essential component of functional genomics and has become a critical resource for molecular biologists conducting strategic, applied, or fundamental research. The following are several conspicuous instances where transgenic technology has been implemented in vegetation. This list is merely illustrative of the possible applications and is not exhaustive.

Imparting resistance to biotic stress

1) Insect resistance - eg: BT crops
2) Virus resistance -eg: rainbow papaya

Imparting resistance to abiotic stress

1) Herbicide resistance - eg: roundup ready soyabean

Improvement of crop yield and quality

1) Extended shelf life of fruits -eg: Flavr savr tomato
2) Improved nutrition -eg: Golden rice
3) Improved coloration -eg: Japanese blue roses

Production of low-cost pharmaceuticals

1) Edible vaccines -eg: Rabies virus g-protein in tomato

2) Essential proteins -eg: Soy bean, maize

Insect resistance (e.g., Bt cotton, the only commercially transgenic crop cultivated in India), nutritional enhancement (e.g., rainbow papaya), virus resistance (e.g., rainbow papaya), herbicide resistance (e.g., roundup-ready crops), and delayed fruit ripening (e.g., flavr savr tomato) are the five most prominent examples that have been utilized across crops and countries. Thus far, the primary focus has been on modifying input traits. However, the forthcoming field-ready exploitation of second-generation transgenic plants is anticipated to enhance output traits. In the future, transgenic plants are anticipated to be utilized in the production of molecules that contribute to the betterment of humanity (Fig. 6). The primary transgenic crops that are cultivated globally consist of cotton, maize, soybeans, potatoes, canola, and brinjal. Numerous nations have implemented these crops for commercial purposes (see Figure 7). A comprehensive compilation of commercially viable transgenic crops and the specific traits that have been engineered is presented in a recent review by Kumar et al. (2020). An illustration from this review is depicted in the figure that follows (Fig. 8).

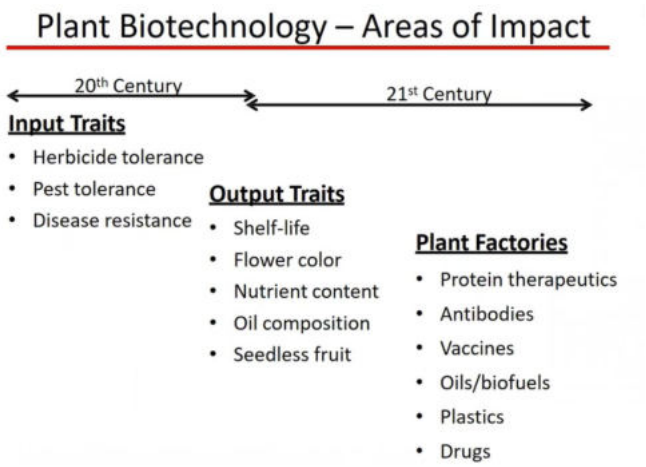


Fig. 6 Traits incorporated in transgenic crops

Crop Name	Countries
Cotton	Argentina,Australia,Brazil,Colombia,India,Mexico,Paraguay,South Africa,USA
Maize	Brazil,Canada,Colombia,Cuba,European Union,Honduras,Paraguay,Philippines,South Africa,USA,Portugal,Vietnam
Soyabean	Argentina,Bolivia,Brazil,Canada,Chile,Costa Rica,Mexico,Paraguay,South Africa,USA,Uruguay
Potato	Canada,USA
Canola	Canada,USA,Australia,Chile
Brinjal	Bangladesh

Fig 7. Major transgenic crops and the countries growing them

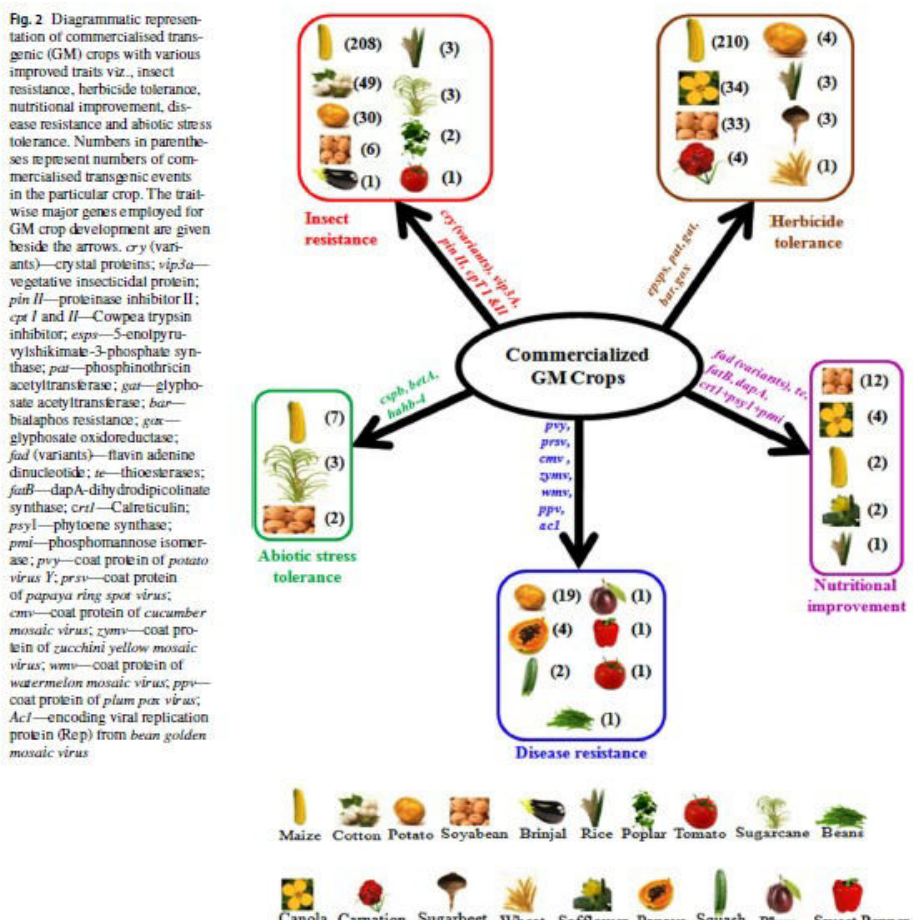


Fig. 2 Diagrammatic representation of commercialised transgenic (GM) crops with various improved traits viz., insect resistance, herbicide tolerance, nutritional improvement, disease resistance and abiotic stress tolerance. Numbers in parentheses represent numbers of commercialised transgenic events in the particular crop. The trait-wise major genes employed for GM crop development are given beside the arrows. *cry* (variants)—crystal proteins; *vip3a*—vegetative insecticidal protein; *pin II*—proteinase inhibitor II; *cpt I* and *II*—Cowpea trypsin inhibitor; *epsps*—5-enolpyruvylshikimate-3-phosphate synthase; *pat*—phosphinothricin acetyltransferase; *gat*—glyphosate acetyltransferase; *bar*—bialaphos resistance; *gox*—glyphosate oxidoreductase; *fad* (variants)—flavin adenine dinucleotide; *te*—thioesterases; *fatB*—dapA-dihydrodipicolinate synthase; *crt1*—Calreticulin; *psy1*—phytoene synthase; *pmi*—phosphomannose isomerase; *pvy*—coat protein of *potato virus Y*; *prsv*—coat protein of *papaya ring spot virus*; *cmv*—coat protein of *cucumber mosaic virus*; *zymv*—coat protein of *zucchini yellow mosaic virus*; *wmv*—coat protein of *watermelon mosaic virus*; *ppv*—coat protein of *plum pox virus*; *Ac1*—encoding viral replication protein (Rep) from *bean golden mosaic virus*

(Source: Taken from Kumar et al., 2020)

Fig 8. Diagrammatic representation of the main target traits manipulated, commercialized transgenic crops, number of events released and the major genes used for imparting the traits

Molecular techniques for identification of transgenic plants

There are several molecular techniques used for testing transgenic plants. These techniques help in confirming the presence of the desired transgene and determining its expression level and stability. Here are some commonly used molecular techniques for testing transgenic plants:

1. Polymerase Chain Reaction (PCR): PCR is a widely used technique to amplify specific DNA sequences. It can be used to detect and confirm the presence of transgenes in plant samples. Specific primers designed to anneal to the transgene sequence are used to amplify the target DNA, and the amplified product is visualized on an agarose gel.

2. Southern Blotting: Southern blotting is a technique used to identify and characterize specific DNA sequences. It involves the transfer of DNA fragments from an electrophoresis gel to a solid support, typically a nylon membrane. The transferred DNA is then hybridized with a labeled probe specific to the transgene sequence, allowing for its detection.

3. Northern Blotting: Northern blotting is similar to Southern blotting but is used to detect and analyze RNA expression. It can be used to determine the expression level of the transgene in plant tissues by hybridizing the transferred RNA fragments with a labeled probe specific to the transgene mRNA.

4. Western Blotting: Western blotting is a technique used to detect specific proteins. It can be used to determine the expression and stability of transgene-encoded proteins in transgenic plant samples. Protein extracts from plant tissues are separated by gel electrophoresis, transferred to a membrane, and probed with a specific antibody against the transgene-encoded protein.

5. Real-time Quantitative PCR (qPCR): qPCR is a highly sensitive technique used to measure the expression level of a gene of interest. It can be used to quantify the amount of transgene mRNA in plant tissues, allowing for the evaluation of transgene expression levels.

6. Next-generation sequencing (NGS): NGS technologies enable high-throughput sequencing of DNA, RNA, or both. It can be used to obtain a comprehensive analysis of the transgenic plant genome and transcriptome, including the identification and characterization of transgene insertions and their expression patterns.

These molecular techniques are commonly used in combination to fully characterize and evaluate the presence, expression, and stability of transgenes in plants.

Methods of GM Seed Testing

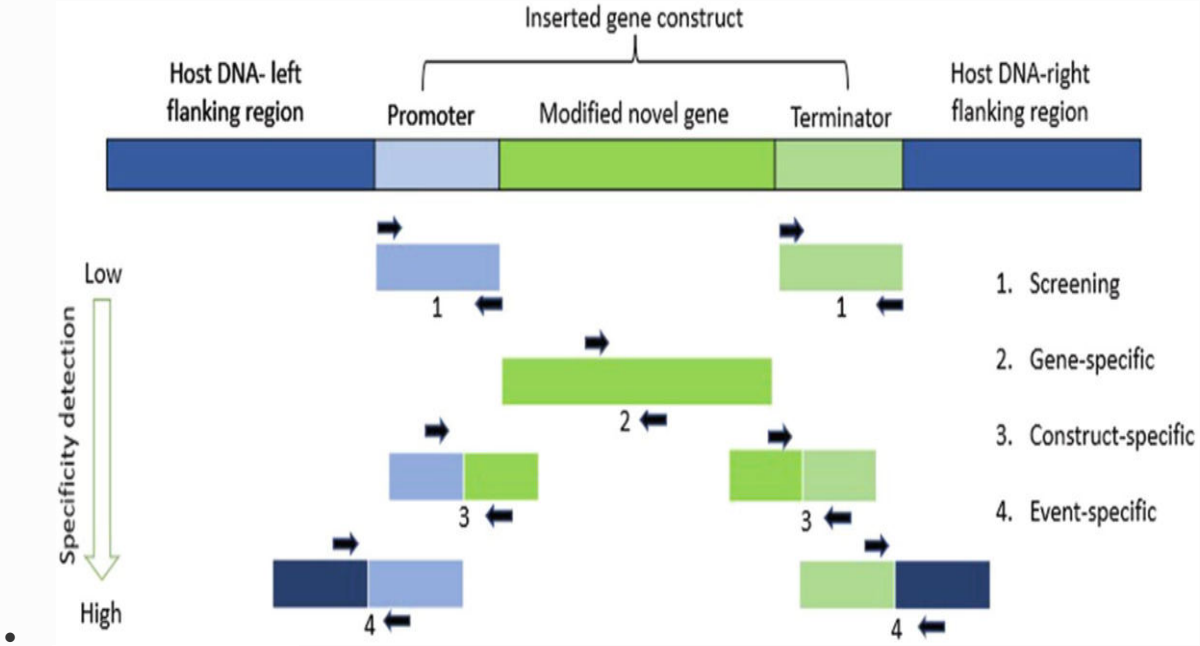
There are four different levels of GM assessment in seed samples as mentioned below:

- GM detection: Performing a seed screening in order to identify the existence of transgenic seedlings. Primers are employed to identify the presence of generic genetic elements (GMOs) in a seed lot by detecting a general genetic element, such as the constitutive promoter or a selection marker, which is commonly present in all GMOs.

- Gene-specific detection: By utilizing primers that are specific to the gene sequences and testing for a particular transgene, it is possible to identify a particular GMO. The identification of a specific transgene being examined in the seeds is suggested by the

presence of amplified fragments.Gene construct-specific detection: Specific gene constructs within a seed batch are identified. Primers specific to sequences of the promoter/terminator and a portion of the gene accomplish this.

- Event-specific detection: Detection of a gene event requires knowledge of the flanking sequences, or the sequence of the host genome that is in close proximity to the targeted gene construct. Utilizing primers designed to detect the unique integration site of a particular GMO, the event is identified.



- Sandra et al. 2023

Take home message from the discussion shall be:

- ✓ Transgenic technology – has become an integral part of plant breeding programmes for precise modification of the genetic architecture. **Genome editing a new tool in the armory.**
- ✓ Transgenic plants are helpful in creating both gain of function and loss of function traits.
- ✓ Transgenic technology has led to greater understanding of the plant life, cellular mechanisms, physiology of traits, complicated gene regulations.
- ✓ We have come a long way – from understanding to transform the organisms to exploiting the technology. Single gene of interest (GOI) to multiple genes (upto 30 kb) transferred and stably inherited. Even minichromosomes could be moved.
- ✓ Moved from input traits to making plants as factories to meet the human needs
- ✓ With so many programmes on integrated genomics – now we have many candidate genes for imparting traits – even the complicated ones (QTLs)

- ✓ Still there is a need to identify suitable regulatory sequences to direct expression of GOI for realizing the intended phenotype in the plant
- ✓ Still there are limitations in realizing transgenic plants at will in crop plants.
- ✓ Social licensing is an issue that needs to be addressed for full exploitation of the TG technology

During the discussion, most of the examples of transgenic crops, traits manipulated and the methods of detection will be dealt in detail with suitable schematic diagrams, genes involved, methods followed and the present status. Examples will be taken from our lab experiences and they will be discussed at length to fix the concepts, principles and procedures. Also, during the course of the interaction class, the issues and concerns raised with respect to commercial release of the transgenic plants into the environment will be discussed.

Further reading:

Kumar, K., Gambhir, G., Dass, A. *et al.* 2020. Genetically modified crops: current status and future prospects. *Planta* **251,** 91-97 (2020). https://doi.org/10.1007/s00425-020-03372-8

Low LY, Yang SK, Kok DA et al. 2018.Transgenic plants: Gene constructs, vector and transformation method. DOI:10.5772/intechopen.79369

Basso MF, Arraes FBM,Grossi-de-Sa M, et al. 2020. Insights into genetic and molecular elements for transgenic crop development. *Front. Plant Sci.* 11:509.doi: 10.3389/fpls.2020.00509

Sandra, N., Ravishankar, K.V. and Chidambara, B., 2023. Molecular Techniques for Testing Genetic Purity and Seed Health. In *Seed Science and Technology* (pp. 365-389). Springer, Singapore.

Reader is recommended to watch the following video for getting a good overview on plant biotechnology.

https://ucdavis.mediaspace.kaltura.com/media/Lecture+20+-+SSC+100+-+11+14+2016/0_jvzav0jq

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Genetic Purity Analysis through Grow Out Test

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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 for 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 plant distance (cm)	Space between rows (cm)	Space between plots (cm)	No. of replications
1.	Wheat, barley, oats	6	2	25	50	2
2.	Pea, cowpea	6	10	45	90	2
3.	Chickpea, green gram, black gram	6	10	30	60	2
4.	Maize	10	25	60	90	2
5.	Hybrid cotton	5	10	45	45	2
6.	Paddy:					
	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 Off-types (%)	**Minimum genetic purity (%)**	**Number of plants required per 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

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Seed Germination: Principles and Procedures

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Seed being a reproductive unit; is the thread of life that assures the survival of all plant species. Seed germination is a unique process in which a series of well organized events enable the expression of the inherent genetic information of the seed in the form of embryo emergence. All seeds must germinate to continue the generational march of plant species. Germination is the first visible symptom of growth and development of embryo, the foundation of which has been laid down during the development and maturation of seed. Furthermore, because of its role in stand establishment, seed germination remains a key in modern agriculture. A fundamental understanding of seed germination is essential for maximum crop production.

Definition

Various definition of seed germination has been proposed, it is important to understand each one of them. Germination has been defined as "a sequential series of morphogenetic events that results in the transformation of an embryo into a seedling" (Singh and Joshi, 2004). Seed physiologist defines seed germination as "the emergence of the radicle through the seed coat". To the seed analyst, 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 favourable condition"(AOSA, 2000). International Seed Testing Association (ISTA) define germination of seed in a laboratory test is "***the emergence and development of the seedling to a stage where the aspect of its essential structures indicates whether or not it is able to develop further into a satisfactory plant under favourable conditions in the field*** (Anon, 2023)". Other considers that germination "is the resumption of active growth of embryo that results in the rupture of the seed coat and the emergence of the young plant" (Copeland and Mc Donald, 2005). This presumes that the seed has been in a state of quiescence or rest, after its formation and development. During this period, the seed remain inactive and has a low metabolic rate. It will remain in this state, until environmental conditions trigger the resumption of growth. All definition s include some measure of seedling development, even though this occurs subsequent to germination event.

Some seeds are capable of germination only a few days later to fertilization and long before their harvesting time; others are dormant and require a extended rest period before germination occur. Depending upon species, this period may last for few only a few days or for as long as several years.

Types of seed germination

Based on the cotyledons or storage organ, two kinds of seed germination occur, not appear to be related with seed structure. These two types are illustrated in fig.1 (*eg*., bean and pea/maize). These seeds are similar in structure, belong to same taxonomic family, their germination patter are different.

Epigeal germination

Epigeal germination (Fig.1) is characteristic of bean seeds. It is considered evolutionary, more primitive than hypogeal germination. During germination, the cotyledons are raised above the ground where they continue to provide nutritive support to the growing point. During root development, hypocotyl elongates in an arch that pull out the cotyledon, enclosing the plumule, through soil and projecting them into air. Afterward, the cotyledon open, plumule growth continues and cotyledons wither and fall to the ground.

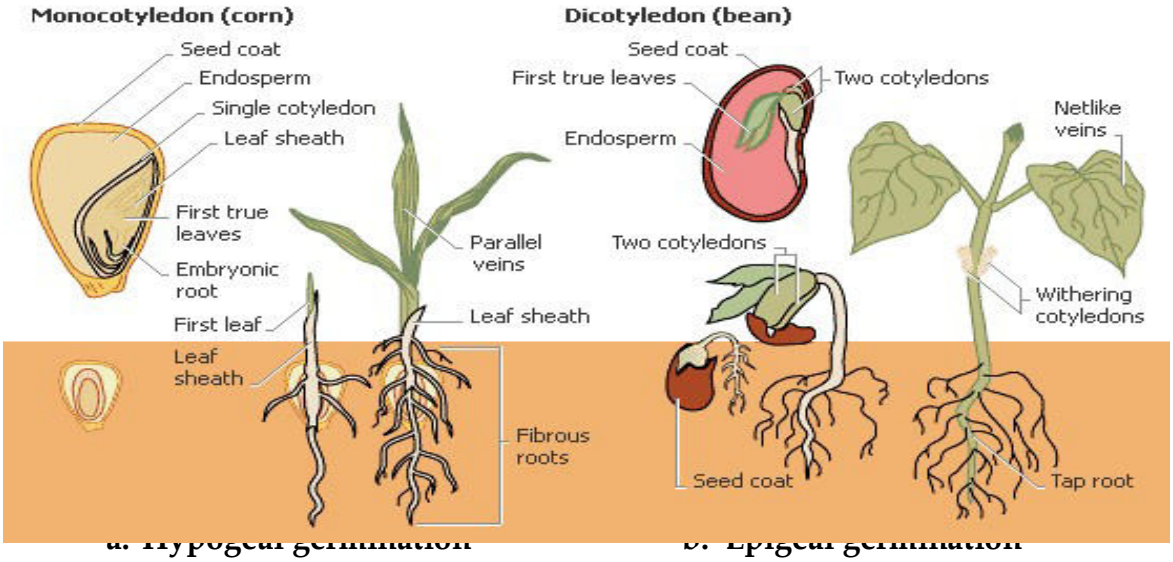

Fig.1. Different types of seed germination

Hypogeal germination

Hypogeal germination (Fig.1) is characteristic of pea seeds, all grasses (as corn) and many other species. During germination, the cotyledons or comparable storage organs remain beneath the soil, whereas, the plumule pushes upward and emerges above the ground. In hypogeal germination, epicotyl elongates. Regardless of their locations (above or below ground), the cotyledons or storage organs continue to provide nutritive support to the growing point throughout germination.

The coleoptile, a temporary sheath enclosing plumule, is associated with hypogeal germination. It provides rigidity and protection to the emerging plumule as it pushes through soil and is exposed to light. The coleoptile stops growing and disintegrates, as the plumule breaks through and continues to grow.

Germination testing

Seed germination is the only accurate and reproducible seed quality test in evaluating the planting value of a given seed lot. Planting value of a seed lot is the ability of seeds to produce normal seedlings and plants. Testing seeds under field conditions, is normally unsatisfactory, irreproducible and unreliable, whereas, under

laboratory conditions, is accurate, uniform and reproducible, as testing environment is controlled. Seeds are tested prior to planting with the objective ***to determine the germination potential of a seed lot, which can in turn be used to compare the quality of different lots and also estimate the field planting value*** (Anon, 2023).

Seed germination results help in:

- Labeling of a seed lot,
- Fixing the prices (marketing) of a seed lot,
- Adjusting the seed rate to achieve the desired field establishment,
- Seed certification (to check, whether a particular seed lot is meeting the minimum seed standards),
- Conservation of plant genetic resources,
- Quarantine,
- Seed law enforcement and
- Seed quality control.

The essential seedling structures

A seedling, depending on the species being tested, consists of a specific combination of the following structures, which are essential for its further development into a satisfactory plant:

- Root system (primary root, in certain cases seminal roots),
- Shoot axis (hypocotyl, epicotyl in certain *Poaceae* (*Gramineae*), mesocotyl, terminal bud)
- Cotyledons (one to several)
- Coleoptile (in *Poaceae* ≅ *Gramineae*)

Germination process

- Imbibitions of water
- Enzyme activity
- Rupture of seed coat and radicle emergence
- Growth and development of seedling

General principles

Germination test must be made from the pure seed, except where testing of seeds by weighed replicate is allowed. The pure seed definition for the species must be applied (Anon, 2023). The pure seed be taken from the pure seed fraction of a purity test. Test a minimal of 400 seeds. Seeds may be in four replicates of 100 seeds each or in eight replicates of 50 seeds each or in 16 replicates of 25 seeds each, depending on the size of seed and/or containers of substrates. The seeds, arranged in replicates, are tested under favourable moisture conditions, in accordance with the prescribed methods. Examine seedling(s), on expiry of prescribed period, counts made of the seedlings, under various categories, for reporting.

Don't treat the seeds, unless desires. If additional tests are undertaken with any pre-treatments, the results and pretreatments be reported under "Other Determinations" on the seed analysis certificate.

Rules for seed testing

- ISTA - International trade
- National(STM) - Seed law enforcement

to achieve accurate, uniform and reproducible results, germination tests are conducted under controlled environment in a lab, following the prescribed procedures

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Requirements for seed germination

- Water/ Moisture
- Temperature
- Light
- Oxygen

Water/ Moisture

High concentration of water is needed at cellular level to start seed germination. Mobilization of food requires hydrolysis to transport food from storage organ to growing tissues. Moisture is supplied through substratum. The moisture should be sufficient throughout the test period. Subsequent watering be avoided, as it increase variability between replicates and between tests.

Quality of water

The water used to moisten the substrate be reasonably free from acid, alkali, organic or other impurities. The pH value of water be within range of 6.0 to 7.5. Generally tap (drinking) water, de-mineralized water, de-ionized water and spring water are common in used for seed germination tests. If distilled water is used, it needs aeration (to enhance O_2); if deionized water is used, use up to 0.5 ppm hardness. Use optimum quantity of water, as too much water allows fungal growth or too less water causes poor seedling growth.

Temperature

Temperatures are those, the seed exposed to on or inside the substrate. It should be as uniform as possible throughout germination apparatus, cabinet or room germinator. Variation in temperature be ± 2^{o}C to that of prescribed temperature. When, alternate temperature is indicated, the lower temperature usually be maintained for 16 hrs and higher temperature for eight hrs. Crop specific temperature is prescribed for individual species in Table 5A of ISTA Rules (Anon 2023).

Light

Seeds of most of the species in field or horticultural crops will germinate either in light or in darkness. However, illumination of the substrate from an artificial source is

generally recommended, as better developed seedlings. Seedlings grown in complete darkness are etiolated and white, and therefore more sensitive to attack by micro-organisms. Besides, certain defects, such as chlorophyll deficiency cannot be detected. Light for seed germination, is generally not required. It is needed by most grasses, some vegetable seeds, flower and tree seeds during seed germination. Cool white nature light is good for seed germination. Light requiring seed, be tested on top of substratum (to prevent filtering of light). Specific requirements for light or darkness are given in last column of Table 5A of ISTA Rules (Anon 2023).

Oxygen

For BP care should be taken that envelopes and towel rolls are loose enough to allow for sufficient air around the seeds. For the same reason the material covering the seeds in sand and organic growing medium should not be compressed.

Equipments or material required

•Containers
•Counting equipments
•Germination apparatus
•Substrates
•Others
.GA_3/ KNO_3
•Petri dishes
•Seed scarifier
•Rakes and scrapers
•Refrigerator/Oven/ Water bath
•Thermometer
•Plastic/Aluminum/ Surgical trays
•Forceps/Rubber band/Glass marking pencil/Pen
•Trolley/Bucket
•Misc lab glassware and supplies

Containers

All kind of containers, made up of plastic, glass, metal, or pottery; transparent or translucent are used, provided there are no toxic emanations. The containers should be clean and free from micro-organisms.

Counting equipments

Any equipment that facilitate counting of seed material. Two types of seed counters are in common use:

- Counting boards
- Vacuum counters

Counting boards: Counting boards are generally used for large seeds *eg.,* maize, beans or pea. It is of approximately equal to the size of the substrate on which seeds are planted. The top consist of a board with 50 or 100 holes, of size similar to that of seed species to be counted. Below this board is another board with another board which serves as bottom. It may be solid to be slide backward and forward or it may contain corresponding holes, which can be closed or open by moving top and bottom board against each other.

Operation of counting boards: Seeds are scattered over the board with holes enclosed underneath. Excess seeds are removed after checking that holes are filled, and there is only one seed in each hole. The holes are opened by sliding the movable board and the seeds fall into place on the substrate.

Vacuum counter: Vacuum counters are generally used for seeds of regular shape and relatively smooth *eg.*, cereals, brassica and alike species. It consists of three main parts: a vacuum system including pipes which don't restrict air flow; a range of counting plates or heads to suits to various seeds, and a vacuum release valve. An ordinary household vacuum cleaner may be used as vacuum system. The heads containing 50 or 100 holes, slightly smaller than substrate, and should have an edge to prevent the seeds from rolling off. The dia of holes be in correspondence with seed size and the vacuum applied.

Operation of a vacuum counter: Seeds are poured evenly over the counting head with the vacuum off. The vacuum is then applied, surplus seeds are removed. A check is made that all holes are filled with only one seed. The head is then placed on germination substrate and the vacuum is released, to allow fall of seeds on substrate. Care be taken, that no selection of seed. Precaution be observed to avoid biased replicates, the counting heads should not be plunged into seeds (as vacuum select light seeds).

Germination apparatus

Different types of germination apparatus are used depending upon the kind and size of seed and facilities available.

The bell jar or Jacobsen apparatus (Copenhagen tanks): This apparatus usually consists of a metallic tray or germination plate upon which filter or towel paper substrates with seeds are is placed. The substrate is kept continuously moist by means of wicks (extended into the underlying water bath). To prevent the drying out of substrates, seed beds are covered with a bell jar, provided with a hole for ventilation. The temperature of water bath is regulated thermostatically. The apparatus is used for all prescribed constant or alternating temperatures (limited by its design). The apparatus is good for light requiring small seeds.

Jones and Cobb apparatus: An inclined rigid plastic or glass tray is placed to support the substratum. Thin blotters on slanted substrate are used for testing of seeds.

Cellulose tissue is used for covering the seed on inclined substratum. A fine mist of water is sprayed over the tissue (Substratum). The apparatus is good for testing speed of seed germination.

Thermo-gradient plate: As the name indicates, the apparatus maintains all temperature ranges between 10^{o} to 40^{o}C; all combination of temperatures; constant and/or alternate temperatures, suitable for most of the field or horticultural crops. The apparatus was designed by Arnold et al (1969) for Crambe abbyssinic. The apparatus is of 30"x50"size, with number of chambers: 25 to 169, having all modern facilities of automatic recording seed germination (Hooked with a computer). The apparatus is good for testing dormant seeds and generally used for dormancy breaking treatments.

Walk-in room germinator or the room germinator: Walk-in room germinator or the room germinator is a modification of the germination cabinet. It is constructed on the same principles, but is large enough, to permit workers to enter and place the tests along either side of a central passage. Alternatively tests may be placed on trolleys which are then wheeled into the room for the test period. Room humidifiers are installed to maintain high level of RH. The room germinators are suitable for blotter paper, filter paper, roll-towel paper, sand, soil/compost and agar media as substrates.

The germination cabinet: The apparatus is a double wall, close cabinet for germinating seeds either in light or dark. Day light germination cabinets are made up of with glass wall (for light). The cabinets maintain darkness, diffuse or direct light. Modern cabinets are insulated and have both heating and cooling systems. The cabinets are suitably designed for constant or alternating temperatures. The temperature is maintained by circulating water or air or both through the cabinet. Water is heated in the bottom cabinet. The germination cabinets are good to maintain temperatures in the range of 5^{o} to 50^{o}C and relative humidity (RH) close to saturation (≥ 95%). The apparatus is equipped with appropriate tray slides (to accommodate trays).

Substrate or growing media

Substrate or growing media for germination tests is a surface or medium, where seed germinate and seedling grow; which provide sufficient pore space for air, for the anchorage of the root system and serve as a moisture reservoir needed for seedling growth. Some of the commonly used growing media are:

- Top of paper (TP)
- Between paper (BP)
- Pleated paper (PP)
- Sand
- Soil or compost
- Agar media (0.7 to 1.0 %)

Top of paper (TP): The seeds are germinated on one or more layer of paper, which are placed

- Either on top of the Jacobsen apparatus,
- Or into transparent boxes or Petri plates, or
- Directly on trays in a cabinet germinators.

The appropriate quantity of water is added at beginning of test. Evaporation is minimized by tightly fitting lid or enclosing the Petri dishes in plastic bags.

Between paper (BP) Method: The seeds are being germinated in between two layers of paper (substrate). This may be achieved by

- loosely covering the seeds with an additional layer of paper,
- placing the seeds in rolled towels (rolls be placed either on flat or upright position),
- Placing of the seeds into folded envelopes.

The substrates are kept in closed boxes, wrapped in plastic bags or wrapped in wax paper. The wrapped substrates are placed directly on trays in a cabinet germinator. The relative humidity in the germinator be maintained near to saturation.

Pleated paper (PP): The seeds are placed in a pleated, accordion-like paper strip with 50 pleats, usually two seeds to a pleat. The pleated strips are kept either in boxes or directly into a wet cabinet, with an flat strip often wrapped around the pleated paper to ensure uniform moisture. This method may be used as an alternative to prescribed TP or BP method.

Sand or organic growing medium: The seeds are placed on the surface of sand or the growing organic medium. Sand may be washed, dried, sterilized and reused. It is used as follows:

- TS (Top of sand)
- Sand or organic medium

TS (Top of sand): The seeds are planted on the surface of sand.

Sand (S) or organic medium: The seeds are planted on a level layer moist sand or the organic growing medium, and covered with 10-20 mm uncompressed substrate (depending on seed size). To ensure good aeration, bottom layer be loosened by raking before planting of seeds.

Sand (S) or organic medium be used as an alternate of paper substrate for evaluation of a diseased seed sample; sometimes to confirm evaluation of seedlings (in cases of doubt), when seedlings show phytotoxic symptoms.

Soil or compost: Soil is generally not recommended as a primary growing medium. However, it may be used as an alternate to growing medium when seedlings show phytotoxic symptoms or evaluation of seedlings is in doubt on paper or sand. It is commonly used for comparative or investigative purposes

Agar medium: Agar medium is good, if seeds are infested with bacteria. It is an expensive and alternate media. Agar concentrations in general use is 0.7 to 1.0 %.

Treatments for promoting seed germination

For various reasons (eg. physiological dormancy, hard seededness and inhibitory substances), a considerable number of seeds remain hard or fresh at the end of standard germination test. Complete seed germination is obtained by retesting the seeds with one or a combination of the treatments given as under. If seed dormancy is suspected, recommended treatments are applied to the original test. Pretreatment and duration of pretreatment must be reported on Seed Analysis Certificate.

Physiological dormancy: In some tree or shrub seeds, where it is known that proportion of seeds will not germinate due to dormancy, a second test incorporating a pretreatment should be run concurrently with the normal test.

Dry storage: For species where dormancy is naturally for short period, the seed samples are stored in a dry place for a short period.

Pre-chilling: The seeds (A few field crops, vegetables, flower spices and medicinal species), in replicates, are placed on moist substrates at low temperature (5^{o} to 10^{o}C) for a period up to 7 days, before they are transferred to specified temperatures for germination. Trees and shrub seeds are pre-chilled at 1^{o} to 5^{o}C for 2 weeks to 12 months prior to seed germination. In such cases quick viability tests are recommended.

Pre-heating: The seeds, in replicates, are heated at temperature (30^{o} to 35^{o}C) with free air circulation for a period up to 7 days, before they are transferred to specified temperatures for germination. In some cases it may be necessary to extend the pre-heating period. For certain tropical and subtropical species, pre-heating temperature of 40^{o}C (Arachis hypogaea) to 50^{o}C (Oryza sativa) is used.

Light: The test be illuminated for at least 8 hrs in every 24 hrs cycle; and during high temperature period, if using alternate temperature. The light intensity be 750 to 1250 lux from cool white lamp. Illumination is recommended for certain tropical and subtropical grasses (eg., Chloris gayana and Cynodon dactylon).

Potassium nitrate (KNO_3): 0.2 per cent KNO_3 solution in water is recommended, to saturate the germination substrates, at the beginning of the seed germination test.

Gibberellic acid (GA_3): 0.01 to 0.1 per cent (or 100 to 1000 ppm) GA_3 solution in water is recommended, to saturate the germination substrates, at the beginning of the seed germination test. When the concentration of GA_3 solution higher than 0.08 per cent is used, the solution be made in a phosphate buffer (1.7799 gm of $Na_2HPO_4.2H_2O$ and 1.3799 of $NaH_2PO_4.H_2O$ in one liter of water). It is recommended for Avena sativa, Hordeum vulgare, Secale cereal and Triticum arestivum.

Sealed polythene envelopes: If a higher proportion of fresh un-germinated seeds are obtained at the end of standard germination test (eg., Trifolium spp), retesting in a sealed polythene envelope of just sufficient size to hold the test is recommended for satisfactory seed germination.

Methods for removing hard seededness

For many species, where hard seeds occur, a special treatment is required. The treatment is applied prior to the commencement of actual germination test. If, it is suspected that the treatment may adversely affect the non-hard seeds, it should be done only on hard seeds isolated at the end of test period. Some of the treatments recommended are:

Hot water treatment or soaking: Seeds with hard seed coats may germinate readily, if soaked in water up to 48 hrs (eg., Acacia spp – plunging seeds in boiling water, volume to its three times; Vigna mungo (Black gram) – plunging seeds boiling water for 5 to 10 minutes). The germination test is commenced soon after soaking.

Scarification: Careful piercing, filling, chipping, and sand papering of seed coat (Saving from embryo damage) is recommended to break seed dormancy due to hard seed coat. The best site for mechanical scarification is part of the seed coat immediately above the tips of the cotyledons.

Acid scarification: Digestion of hard seed coat in concentrated H_2SO_4 is effective in some species (eg., Macrotptilium spp). The seeds are soaked in the acid until the seed coat becomes pitted. Digestion may be rapid or takes approx one hr, seeds be examined every few minutes. Seeds be washed in running water soon after digestion, before planting for germination test. In Oryza sativa, seeds are soaked in 1N HNO_3 for 24 hrs (soon after preheating at 50^{o}C for a period up to 7 days).

Method of removing inhibitory substances

Chemical substances in the seed coat, inhibiting germination, may be removed as under:

Prewashing: Naturally occurring substances in the seed coat or pericarp, inhibiting germination, may be removed by washing the seeds in running water at temperature around 25^{o}C, before germination test is made (eg., Beta vulgaris).

Removal of outer structure: Germination in certain species is promoted by removing the outer structures of seeds eg., involucres of bristles or lemma and palea in certain Poaceae or Gramineae.

Duration of test

The duration of test on individual species is given in ISTA Rules (Table 5A) Anon, 2023. The duration of the treatment required for breaking dormancy before or during the test is not accounted as a part of germination test period. If the maximum

germination has been obtained before the end of prescribed test period, a test may be terminated earlier.

The time for first count is approximate, but must be sufficient to allow the seedlings to reach a stage of development, which allows it for accurate evaluation. All essential structures of seedling must be visible and grown to full extend for accurate evaluation. The times indicated in Table 5A refers to highest temperatures. If tested at lower temperature, the first count be postponed. Sometime first count may be omitted, for tests in sand lasting not more than 7 to 10 days. Intermediate counts to remove seedlings which are sufficiently well developed are recommended to make the counting easier, and may be left to the discretion of the seed analyst.

Choice of method

Choice of alternate methods is given in Table 5A of ISTA Rules (Anon, 2023). Any combination of substrate and temperature may be used. The choice of method largely depends on facilities and experience of seed testing laboratory and to some extent on the provenance and condition of the samples. If a sample does not respond satisfactorily, re-test is suggested by one or more alternative methods.

Procedure

A minimum of 400 (in replicates) seeds be taken at random from pure seed fraction. Seeds should be spread equidistantly, on any chosen suitable substrate (BP or TP or PP or TS or S or Soil or Compost or Agar). Tests (The roll towels/Petri plates or sand boxes) be suitably transferred into germination cabinet or walk-in-room germinator. Seedlings should be evaluated on expiry of test period.

Seedling evaluation

Seedlings which have reached to a stage when all essential structures can be accurately assessed must be readily evaluated on primary substrates. Every seedling must be evaluated based on essential structures (sufficiently developed to permit detection of any abnormality). A retest is recommended, on an alternate medium, if seedlings on a given test cannot be readily evaluated. Seedlings be recorded under the prescribed categories of seedlings/seeds.

Categories of seedlings/seeds

Seeds at the end of standard germination tests (in good quality soil under favorable conditions of moisture, temperature and light) are classified into following categories:

- Normal seedlings
- Abnormal seedlings
- Multigerm seed units
- Ungerminated seeds

Normal seedlings

Seedlings show the potential for continued development into satisfactory plants, when grown in good quality soil under favorable conditions of moisture, temperature and light. Seedling must conform to one of the following categories:

Intact seedlings: Seedlings with all essential structures well developed, complete in proportion and healthy.
Seedling with slight defects: Seedling showing slight defects in essential structures provided they show a satisfactory and balanced development comparable to that of intact seedlings.
Seedling with secondary infection: Seedlings have been affected by fungi or bacteria from sources other than the parent seed, provided they conformed to one of the above.

Abnormal seedlings
Seedlings do not show the potential to develop into normal plant, when grown in good quality soil and under favorable conditions of moisture, temperature and light. Abnormal seedling must conform to one of the following categories:
Damaged: Seedling with one of the essential structures is missing or so badly damaged that balanced development of the seedling is not expected.
Deformed or unbalanced seedlings: Weak development of a seedling or in which essential structure is deformed or out of proportion.
***Decayed seedlings*:** Seedling with one of the essential structure is either diseased or decayed (result of primary infection), normal development of a seedling is prevented.
Seedling abnormalities: One or more severe defects in the seedling renders it abnormal:

- the primary root or seminal root,
- the hypocotyl, epicotyl, mesocotyl,
- the cotyledons (50%),
- the primary leaves (50%),
- the terminal bud and surrounding tissue, and
- the coleoptile and the first leaf.

Multigerm seed units
Type of seed units, produce more than one seedling, is of following categories:
Seed units contain more than one true seed: Thus seed units produce more than one seedlings {*eg.,* multiple seed units in *Dactylis, Festuca* and *Lolium*; unseparated schizocarps of *Apiaceae*; cluster of *Beta vulgaris* (*Umbelliferae*)}.
True seed containing more than one embryo: Polyembryony in *Tectona grandis*. In such cases, sometimes, one of the seedlings is weak or spindly, but both are of normal size.
Fused embryo: Two seedlings fused together, produced from one seed.

Un-germinated seeds
Seeds failed to germinate at the end of test period, when tested in good quality soil and under favorable conditions of moisture, temperature and light, are of following categories:
Hard seeds: Hard seededness is a form of dormancy. It is common in species of *Fabaceae* (*Leguminosae*); also occur in other families. Seeds that do not imbibe water are counted and reported as such.
Fresh seeds: Fresh seeds are able to imbibe water, but the germination process is blocked.

Dead seed: Seeds are soft, discolored, and frequently moldy; show no sign of seedling development.

On request, un-germinated seeds are further subdivided into:
Empty seeds: Seeds which are completely empty or contain only residual tissue.
Embryo less seed: Seeds, which contain fresh endosperm or gametophyte tissue, in which there is neither embryonic cavity nor embryo.
Insect-damaged seed: Seeds with insect larvae or show evidence of insect attack, affecting the seed germination.

Retesting

If result of a test is unsatisfactory, must not be reported, and a second test must be made either in similar conditions or using alternate conditions. A rest is recommended under the following circumstances:
Dormancy persistence: When seed dormancy is suspected, method be applied to break the seed dormancy. The best result achieved must be reported. Method should also be reported on Seed Analysis Certificate.
Phytotoxicity or spread of fungi or bacteria: If the results are unreliable due to Phytotoxicity or spread of fungi or bacteria, a retest must be made using one or more alternative methods, preferably in sand or organic growing medium. If required, distance between seeds be increased. The best result achieved must be reported. Method should also be reported on Seed Analysis Certificate.
Wrong evaluation of seedlings or counting: If there is difficulty in deciding the correct evaluation of a number of seedlings, a rests must be made using one or more alternative methods, preferably in sand or organic growing medium. The best result achieved must be reported. Method should also be reported on Seed Analysis Certificate.
Error in test conditions: If there is error in test conditions, seedling evaluation or counting, a rests must be made using the same method. The result of retest must be reported on Seed Analysis Certificate.
Replicates are out of tolerance: If the range for the 100-seed replicates exceeds the maximum tolerated range, a rests must be made using the same method; if the second result is compatible with the first (the difference between two test results is within the tolerance limit) the average of the two tests must be reported on Seed Analysis Certificate.

If the second test result is not compatible with the first and the difference exceeds the prescribed tolerance, a third test using the same method must be made. The average of compatible results be reported.

Calculation and reporting of results

The result of the germination percentage is calculated as average of four 100-seed replicates (sub-replicates of 50 or 25 seeds are combined into 100 seed replicates). Results are expressed in percentage by number of normal seedlings. The percentage is calculated to the nearest whole number (0.5 is taken to the higher number). Likewise, %ages of abnormal seedlings, hard, fresh and dead seeds is calculated. The sum of the percentage of normal, abnormal seedlings and un-germinated seeds must be 100. The

average of normal seedlings, from four replicates of 100 seedlings each, as germination percentage be reported on. If the result is nil, for any of these categories, reported as "0", instead of leaving the appropriate column blank on Seed Analysis Certificate.

Following items are must to be entered on Seed Analysis Certificate

- Duration of test.
- Percentage calculated, nearest to whole number of normal seedlings.
- Substrate and temperature used.
- Any special treatment used for promoting the seed germination.

Upon request

- The result of any additional test,
- The viability of un-germinated seeds and method used to determine it,
- Categories of un-germinated seeds,
- Number of normal seedlings by 100 units, in multi-germ seed units, and
- Proportion of units producing one, two or more than two normal seedlings.

Precautions during seed germination test

The growing medium should remain moist enough during entire test period. Excess moisture be avoided. Temperature of germination apparatus be maintained within the prescribed range of 10^{o} to 35^{o}C. If using the alternate temperatures; under Rules (ISTA) first temperature is always a low temperature; keep the low temperature longer *ie.,* for 16 hrs and high temperature for short duration *ie.,* for 8 hrs. Count the seedlings, when the prescribed period is over from start of seed planting.

References

- Anonymous (2023) International Rules for Seed Testing Seed Science and Technology: 5-i to 5-xxxxxviii
- AOSA (2000) Association of Official Seed Analysts
- Arnold *et al* 1969 Thermo gradient Plate
- Copeland LO and Mc Donald MB (2005) Principles of Seed Science and Technology
- Singh BD and Joshi AK (2004) Seed Science and Technology

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Advance Techniques for Estimation of Moisture Content in Seed Sample

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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 characterize 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 is 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 have 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. Desiccators 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 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.

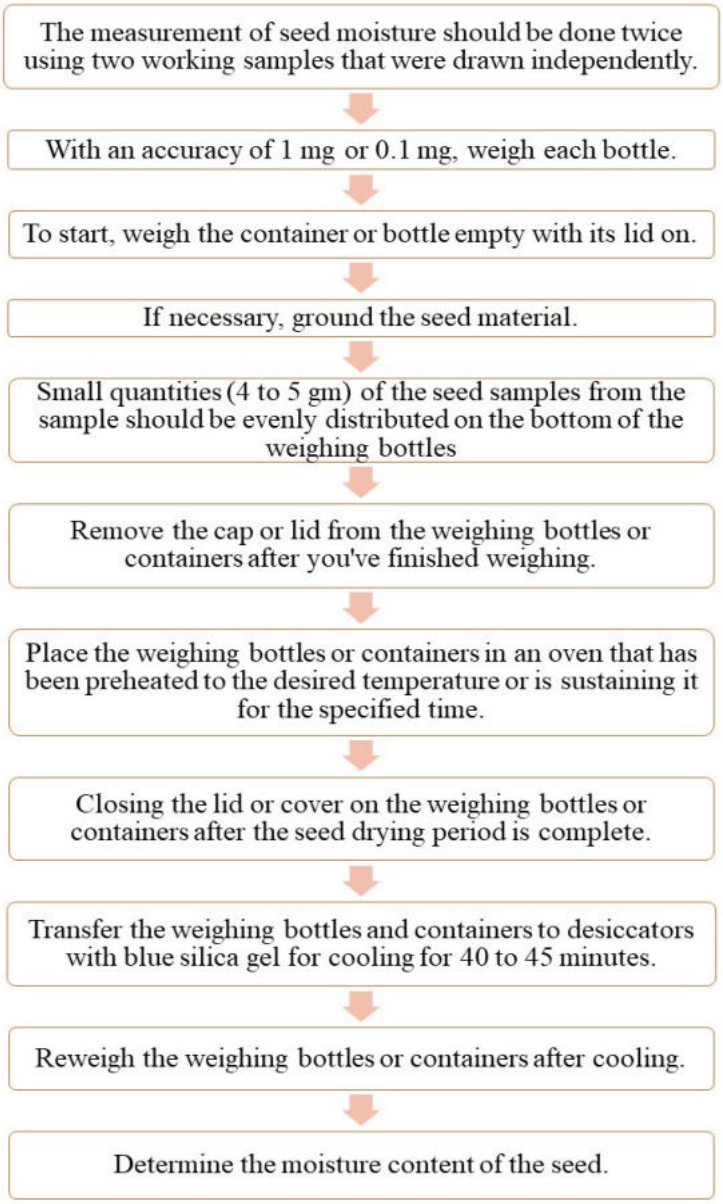
Sample

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. Desiccators
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:

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

Where,

M_1 is the weighing bottle's or container's weight in grams with the cover on.

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

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 (Source: ISTA)

Sr. No.	Species	Grinding/cutting	Method to be used	Drying at high temperature (h)	Pre-drying requirement
1	*Agrostis spp.*	No	High	1	–
2	*Allium spp.*	No	Low	–	–
3	*Alopecurus pratensis*	No	High	1	–
4	*Anethumgraveolens*	No	High	1	–
5	*Anthoxanthumodoratum*	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 pureum*	Coarse	High	1	To 17 % moisture content or less
48	*Medicago spp.*	No	High	1	–
49	*Megathyrsus maximus*	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 lycopersicum*	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
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. Ltd*.

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

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Determination of Seed Viability

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Seed Viability

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

Or

Seed viability refers to state of aliveness

Objectives of seed viability

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

Factors affecting seed viability

1. Internal factors

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

2. Genetic factor

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

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

Seeds of other species are characteristically short lived; these include vegetables such as lettuce, onion, and parsnip and also agronomic crops such as Rye. Generally seed species possessing high oil content do not store as well as those with low oil content. For ex, whole wheat seeds contain only about 3% oil, but their embryo portion has about 27% oil. Seeds of different species may also be chemically similar but have different storability due to differences in genetic potential. For example, Chewings Fescue and annual rye grass seeds are similar in appearance and chemical composition; however rye grass seeds have much better storability under comparable conditions. Genetic differences in storage potential are not limited to seeds of different species, It also occur among cultivars. The bean cultivar black Valentine stores better than Brittle wax (Toole and Toole 1953). However the environment strongly alters the genetic potential for seed longevity.

Relative humidity and temperature

Temperature

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

Seed Moisture

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

Viability tests

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

1. Standard germination test

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

Steps of germination test

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

Seedling evaluation:

- CONCEPT: Evaluation should be done only after all essential structures are fully expressed & evaluate as NS, AS, HS, FUG & dead seeds

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

2. Tetrazolium test

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

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

Objectives:

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

Equipments and chemicals required:

a. One percent solution (W/V) of 2, 3, 5 Triphenyl tetrazolium chloride (TZ) or bromide.
b. Potassium dihydrogen phosphate.
c. Disodium hydrogen phosphate.
Conditioning Media: Blotter, paper towel or beaker.
Cutting or piercing devices: Razor blade, dissecting knives and needles.
Staining dishes: Watch glasses/petridishes.
Magnifying devices: Hand lens and stereoscopic microscope.

Preparation of buffer solution

Solution 1 – dissolve 9.078 g KH_2PO_4 in 1000 ml water
Solution 2 – dissolve 11.876 g Na_2HPO_4 in 1000 ml water
Mix 400 ml of solution 1 with 600 ml of solution 2 to get a liter buffer solution of neutral pH.
To get 1% of TZ solution, dissolve 1 g of TZ salt in 100 ml of buffer solution. (The one percent solution is used for seeds that are not bisected through the embryo, while the 0.1 percent solution is used for seeds in which the embryo is bisected. Other low

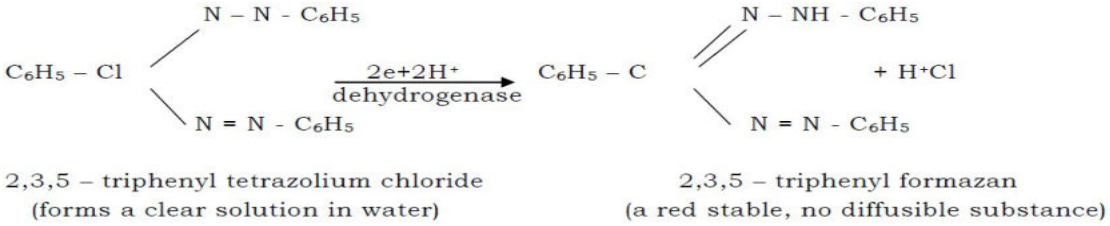
concentration such as 0.2 percent and 0.5 percent are some time used instead of 0.1 percent solution).

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

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

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

The reaction as follows:



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

1. On the basis of staining of embryo

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

2. Assessment on the basis of cotyledon

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

3. Assessment on the basis of necrosis

a. Unstained tissue at the attachment of the embryo-non viable.
b. Unstained tissues are away and are not connected with embryo-viable.

4. Assessment on the basis of color intensity
a. Dark red – vigours seed.
b. Pink color -weak seed.
c. Dark red fractured- non viable.

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

B. Non germinable seeds f cereals
a. Whole embryo unstained.
b. Scutellum node unstained.
c. Major area of coleoptiles unstained.

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

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

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

Advantages of TZ:
1. Quick estimate of viability can be obtained (within 12-20 hrs.)
2. When the seed is dormant or very slow in germination, a viability test is extremely useful.
3. Seeds are not damaged (in dicot only) in analysis, therefore they could be germinated.
Disadvantages of TZ:
1. It is difficult to distinguish between normal and abnormal seedlings.
2. It does not differentiate between dormant and non dormant seeds.

3. Excised embryo test
- The excised embryo test is similar to germination tests in that it measures the quality of the seed by their actual germination.

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

4. Fast green test

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

5. Conductivity test

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

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The Importance and Use of Tolerances In Seed Testing

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Primary risk in agriculture arises when seeds lacking the capacity to yield an abundant crop of the desired cultivar are sown. To mitigate this risk, seed testing has been developed to assess seed quality before planting. The widespread movement of seeds across regions, facilitating significant seed trade development, has heightened farmers' awareness of seed quality. Due to the inherent variability in seeds and their movement between regions, test results must be reproducible not only within individual laboratories but also between them. Therefore, meticulous care must be taken in the procedures used, the proper use of equipment, and the exercise of the best judgment possible in making various evaluation decisions in seed testing. Since seeds are living commodities, no two samples taken from the same seed bag or lot are likely to be identical. Although testing an entire seed lot would ascertain its true value, this is neither feasible nor practical. Thus, in seed testing, the quality of the lot must be inferred from a representative sample. Four major situations may arise, putting pressure on seed testing laboratories to ensure the reproducibility of their results.

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

For the above situations and to ensure that analysis conducted within the laboratory is accurate, it in necessary at the part of the analyst to make sure that the result being reported is valid/reproducible.

The application of an appropriate statistical method to test the results of seed testing enables the analyst to determine the validity of results within a calculated range of limits, the amount of this range in called the **'Tolerance'**.

For seed quality determination, replicated test is conducted from same sample or different samples drawn from the same lot and replicated tests are conducted in one laboratory or different laboratories. It is generally observed that the test results may not be the same. The differences among the result are compared with non-significant permissible value supported with the statistical evidence or calculated range of limits. In seed testing, this maximum non-significant calculated range of limit or expected variation is called **"Tolerance Number"** and a series of such tolerance numbers is called **'Tolerance Table'**.

Tolerance tables are used for testing the significance of the precise estimates for (a) to describe the seed quality (b) to decide if the estimate agrees well enough with another estimate or specification. In other words the object of using tolerances is to provide a

means of assessing whether or not the variation within the test results or between the tests is sufficiently wide to raise doubt about the accuracy of results.

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

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

C. Where to Apply Tolerances:
C.1. Within Seed Testing Laboratory:
Before release of seed analysis reports, a seed analyst has to make sure about the accuracy and reproducibility of results. Several situations normally arise in the seed-testing laboratory when tolerance should be applied to decide the validity of the tests. Some of them are:
(a) Germination test: To decide whether the replicate wise performance of a germination test is comparable or retest- is needed.

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

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

(d) Tetrazolium test: To decide whether the replicate wise performance of a Biochemical/ TZ test is comparable or retest- is needed.

(e) seed vigour test: To decide whether the replicate wise performance of a various vigour tests (conductivity, Accelerated aging, radicle emergence)is comparable or retest- is needed.

C.2. For Certification:
(a) The Central Seed Committee (CSC), Government of India has prescribed the minimum seed certification standards for various crops. As a practice these standards are also taken

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

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

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

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

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

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

C.3. Outside seed testing laboratory:
(a) Seed law enforcement- Label prescription:
Under seed law enforcement, the information given on the label affixed/attached on each container of the lot may be above the prescribed seed standards but under prevailing practice in India the information about seed quality attributes given on the label is the same as per prescribed seed standards for each seed quality attribute. In several other countries the seller normally sells seeds at higher price when label information is above the prescribed seed standards. In such situations the analysis results of seed samples drawn by seed inspectors are compared with the actual information given on the label and tolerances are applied to determine if label information is close enough to analysis results.

(b) Seed law enforcement -Seed standard prescription:
The central seed committee has prescribed crop wise minimum seed certification standards class wise for labeling seeds. At present the seed certification standards are as

such taken for labeling, therefore information on the labels given as per prescribed standards even though the sample might be recording higher germination and purity than the standards. In other words for law enforcement, tolerances are applied whether or not the lot is at least equal to the prescribed minimum limit given on the label.

D. How to Use Tolerance Table:

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

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

(a) How to Use For Purity Analysis Results:

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

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

Average analysis of two half		**Tolerance for differences between**	
samples or two whole sample		**Half working sample**	**Whole working sample**
1	2	3	4
99.95-100.00	000-0.04	0.23	0.16
99 90-99 94	005-009	0.34	0.24
99.85-99.89	0.10-0.14	0.42	0.30
99.80-99.84	0.15-0.19	0.49	0.35
99.75-99.79	0.20-0.24	0.55	0.39
99.70-99.74	0.25-0.29	0.59	0.42
99.65-99.69	0.30-0.34	0.65	0.46
99.60-99.64	0.35-0.39	0.69	0.49
99.55-99.59	0.49-0.44	0.74	0.52
99.50-99-54	0.45-0.49	0.76	0.54
99.40-99.49	0.50-0.59	0.82	0.58

99.30-99.39	0.60-0.69	0.89	0.63
99.20-98.29	0.70-0.79	0.95	0.67
99.10-99.19	0.80-0.89	1.00	0.71
99.00-99.09	0.90-0.99	1.06	0.75
98.75-98.99	0.00-1.24	1.15	0.81
98.50-98.74	1.25-1.49	1.26	0.89
98.25-98.49	1.50-1.74	1.37	0.97
98.00-98.24	1.75-1.99	1.47	1.04
97.75-97.99	2.00-2.24	1.54	1.09
97.50-97.74	2.25-2.49	1.63	1.15
97.25-97.49	2.50-2.74	1.70	1.20
….*	….	….	….
….	….	….	….
….	….	….	….
82.00-83.99	16.00-17.99	3.90	2.76
80.00-81.99	18.00-19.99	4.07	2.88
78.00-79.99	20.00-21.99	4.23	2.99
76.00-77.99	22.00-23.99	4.37	3.09
74.00-75.99	24.00-25.99	4.50	3.18
72.00-73.99	26.00-27.99	4.61	3.26
70.00-71.99	28.00-29.99	4.71	3.33
65.00-69.99	30.00-34.99	4.86	3.44
60.00-64.99	35.00-39.99	5.02	3.55
50.00-59.99	40.00-49.99	5.16	3.65

*. …Indicates the other values in the series

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

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

*. …Indicates the other values in the series

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

Av. (2 Est.	3	…	9-10	…	96-102	…	199-20	…	301-31	…	395-40
Max Tol D	5	…	9	…	28	…	40	…	49	…	56

*. …Indicates the other values in the series

Examples of Use of Tolerance Table for Purity Analysis Results:

(i) To ensure accuracy in a laboratory, it is often desired that the submitted sample is divided first into working samples. Two different seed analysts then analyze each working sample. Suppose purity percentages of the whole working samples in two tests (by the two

analysts) were **98.5** and **96.2**. The question thus arises whether their difference is acceptable. To determine, this add the two values (**98.5+96.2=194.7)** and calculate the mean (**194.7/ 2 = 97.35).** Now in column **1** of table **1** find the range that compares with the average figure; it is **97.25-97.49**. The tolerance value given in column **4** of the table is **1.20.** The difference between the two working sample is **98.5-96.2=2.3**. Since this difference is more than the tolerance the results are not equal, comparable or acceptable; hence' fresh test has to be conducted by drawing another working sample.

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

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

(b) to Use For Germination Test How Results:

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

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

Average percent germination		**No. replicates of 100 seeds**		
		4 rep.	**3 rep.**	**2 rep.**
1	**2**	**3**	**4**	**5**
99	2	5	4	-
…	…	…	…	…
97	4	7	6	5

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

*. …Indicates the other values in the series

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

Average percent Germination More than 50 Percent %	50 Percent or less than	**Tolerance**
1	**2**	**3**
98-99	2-3	2
95-97	4-6	3
91-94	7-10	4
85-90	11-16	5
77-84	17-24	6
60-76	25-41	7
51-59	42-50	8

*. …Indicates the other values in the series

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

Average Percent More than 50 Percent	50 Percent or less than 50	**Tolerance**

1	**2**	**3**
99	2	2
97 To 98	3 To 4	3
94 To 96	5 To 7	4
91 To 93	8 To 10	5
87 To 90	11 To 14	6
82 To 86	15 To 19	7
76 To 81	20 To 25	8
70 To 75	26 To 31	9
60 To 69	32 To 41	10
51 To 59	42 To 50	11

*. …Indicates the other values in the series

Examples of Use of Tolerance Table for Germination Test Results:

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

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

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

E. Use of Tolerances Under Seed Law Enforcement Programme:

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

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

(a) Table no.-7 is meant for comparing the laboratory's results of purity test with the details on label. To use this table both the laboratories results and the details on label should be in percentage by weight.

(b) Table no.-8 is used to compare laboratory's results on other crop seed and weed seed by number per unit weight with the details on label. To use this table both the laboratory's results and details on label should be in number per unit weight.

(c) Table no.- 9 is for comparing germination results reported by the laboratory with the details on table.

(d) Table no.-10 and 11 are meant for comparing laboratory's purity analysis results with the minimum standards prescribed.

(e) Table no.- 12 is to be used for comparing laboratory's germination results with the minimum seed standards prescribed.

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

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

Weed Seed (Maximum)	0.1	0.1	Trace
Germination (Minimum)	94	85	80

(f) From the above (a to e), two points emerge:

(i) Whether the labeling information provided is valid or not even after applying tolerances,

(ii) If even after applying the tolerances the information given on the label does not come in conformity,

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

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

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

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

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

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

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

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

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

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

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

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

(i) Table-10 is meant for any purity component in percentage by weight. The procedure is:

Work out the difference between the laboratory result and the standard prescribed; for example, pure seed.

Laboratory result: 97.0 Standard : 98.0 Difference : 1.0

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

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

(iii) For germination Table 12 is used. The difference between the laboratory germination and the standard prescribed in worked out. For example, it is (85-80)=5. Taking the number of replicates as four of 100 seeds each the total number of seeds in 400. Fit the

standard prescribed *viz.* 85% suitably in Table-12 column C. The tolerance is 6 while the actual difference is only 5. Thus the laboratory result is in conformity with seed standards and seed lot can be soled as standard seed.

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

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

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

Average of 2 estimates		**Non Chaffy Seeds**	**Chaffy Seeds**
A	B	C	D
99.95-100.00	0.00-0.04	0.12	0.14
…..	…..	…..	…..
99.50-99.54	0.45-0.49	0.44	0.52
99.40-99.49	0.50-0.59	0.47	0.56
…..	…..	…..	…..
99.00-99.09	0.90-0.99	0.61	0.72
98.75-98.99	0.00-1.24	0.66	0.78
…..	…..	…..	…..
97.00-97.24	2.75-2.99	1.02	1.21

96.50-96.99	3.00-3.49	1.08	1.28
…..	…..	…..	…..
95.00-95.49	4.50-4.99	1.28	1.51
94.00-94.99	5.00-5.99	1.37	1.62
…..	…..	…..	…..
90.00-90.99	9.00-9.99	1.76	2.07
88.00-89.99	10.00-11.99	1.88	2.20
…..	…..	…..	…..
70.00-71.99	28.00-29.99	2.72	3.20
65.00-96.99	30.00-34.99	2.81	3.30
60.00-64.99	35.00-39.99	2.90	3.41
50.00-59.99	40.00-49.99	2.98	3.50

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

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

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

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

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

Average percent Germinatior		**4Tests**	**3Tests**	**2Tests**
A	**B**	**C**	**D**	**E**
99	2	2	2	1
98	3	3	2	2

97	4	3	3	2
96	5	3	3	3
95	6	4	3	3
94	7	4	4	3
93	8	4	4	4
92	9	5	5	4
91	10	5	4	3
90	11	5	5	4
89	12	5	5	4
88	13	6	5	4
87	14	6	5	4
86	15	6	6	5
..	..	..	..	..
78	23	7	7	6
..	..	..	..	..
75	26	8	7	6
67	34	8	8	6
..	..	..	..	..
63	38	8	8	6
62	39	9	8	6
60	41	9	8	6
59	42	9	8	7
..	..	..	..	..
51	50	9	8	7

Based on 5% probability
Hand book of Tolerances and Measures of Precision for seed Testing, Table G2,
Table -10 Tolerance for comparing laboratory results of purity analysis with the standard prescribed (when both the laboratory result and the standard are in percentage by weight)

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

Only the 5% probability column is used.

Hand book of Tolerances and Measures of Precision for seed Testing, Table P15.

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

A	0.00	0.05	0.10	0.15	..	0.80	0.85	..	1.00	2.00	3.00
B	1	1	2	2	..	3	4	..	4	6	7
A	4.00	5.00	6.00	7.00	8.00	9.00	10.0	11.0	12.0	13.0	14.0
B	9	10	11	13	14	14	16	18	19	20	21
A	15.0	16.0	17.0	18.0	19.0	..	40	41	42	..	500
B	23	24	25	26	27	..	52	53	53	..	538

A= Standard **B= Rejected**

5% Probability level is shown.

Hand book of Tolerances and Measures of Precision for seed Testing, Table F, only

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

Average percent Germinatior		**4Tests**	**3Tests**	**2Tests**
A	**B**	**C**	**D**	**E**
99	2	1	1	2
98	3	2	1	3
97	4	2	1	3
96	5	2	2	4
95	6	3	2	4
94	7	3	2	4
93	8	3	2	5
92	9	3	2	5
91	10	4	3	6
..	..	..	..	..
86	15	5	3	7
..	..	..	..	..

82	19	5	4	7
81	20	5	4	8
..	..	..	..	..
79	22	6	4	8
..	..	..	..	..
75	26	6	4	9
..	..	..	..	..
70	31	7	5	9
69	32	7	5	10
..	..	..	..	..
59	42	7	5	11
..	..	..	..	..
57	44	8	5	11
..	..	..	..	..
51	50	8	5	11

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

(c) To Use For Tetrazolium Test How Results:

Table .13 Maximum tolerated range between four replicates of 100 seeds in one tetrazolium test (two-way test at 2.5 % significance level)

Average viability (%)		**Maximum range**
1	2	3
99	2	5
98	3	6
97	4	7
96	5	8
95	6	9
93–94	7–8	10
91–92	9–10	11

89–90	11–12	12
87–88	13–14	13
84–86	15–17	14
81–83	18–20	15
78–80	21–23	16
73–77	24–28	17
67–72	29–34	18
56–66	35–45	19
51–55	46–50	20

.This table indicates the maximum range (i.e. difference between highest and lowest) in percentage of viable seeds tolerable between replicates, allowing for random sampling variation only at 0.025 probability. To find the maximum tolerated range in any case calculate the average percentage, to the nearest whole number, of the four replicates: if necessary, form 100-seed replicates by combining the subreplicates of 50 or 25 seeds which were closest together in the incubator. Locate the average in column 1 or 2 of the table and read off the maximum tolerated range opposite in column 3.

Table .14 Tolerances for tetrazolium viability tests on the same or a different submitted sample when tests are made in the same laboratory each on 400 seeds (two-way test at 2.5 % significance level)

Average viability (%)		**Maximum range**
99	2	5
98	3	6
97	4	7
96	5	8
95	6	9
93–94	7–8	10
91–92	9–10	11
89–90	11–12	12
87–88	13–14	13
84–86	15–17	14
81–83	18–20	15
78–80	21–23	16
73–77	24–28	17
67–72	29–34	18
56–66	35–45	19
51–55	46–50	20

Table .15 Tolerances for tetrazolium viability tests on two different submitted samples in different laboratories each on 400 seeds (one-way test at 5 % significance level)

Average viability (%)		**Maximum range**
1	2	3
99	2	4
98	3	5
97	4	6
95–96	5–6	7
93–94	7–8	8
91–92	9–10	9
89–90	11–12	10
86–88	13–15	11
82–85	16–19	12
78–81	20–23	13
73–77	24–28	14
65–72	29–36	15
51–64	37–50	16

(d) To Use For vigour Test Results:

Conductivity test:

Table .16 Maximum tolerated range between four replicates within a conductivity test (5 % significance level)

Average conductivity (µS cm^{-1} g^{-1})		**Maximum range**
From	**To**	
1	2	3
10	10.9	3.1
11	11.9	3.3
12	12.9	3.6
13	13.9	3.8
14	14.9	4.1
15	15.9	4.3

16	16.9	4.6
17	17.9	4.8
18	18.9	5.1
19	19.9	5.3
20	20.9	5.5
21	21.9	5.8
22	22.9	6.0
23	23.9	6.3
24	24.9	6.5
25	25.9	6.8
26	26.9	7.0
27	27.9	7.3
28	28.9	7.5
29	29.9	7.8
30	30.9	8.0
31	31.9	8.3
32	32.9	8.5
33	33.9	8.8
34	34.9	9.0
35	35.9	9.3
36	36.9	9.5
37	37.9	9.8
38	38.9	10.0
39	39.9	10.3
40	40.9	10.5
41	41.9	10.8
42	42.9	11.0
43	43.9	11.3

44	44.9	11.5
45	45.9	11.8
46	46.9	12.0
47	47.9	12.3
48	48.9	12.5
49	49.9	12.8
50	50.9	13.0
51	51.9	13.3
52	52.9	13.5
53	53.9	13.8

. Tolerances for two conductivity tests on the same submitted sample when tests are made in the same laboratory (two-way test at 5 % significance level)

This table indicates the maximum range (i.e. difference between highest and lowest) in conductivity reading that is tolerable between replicates. To find the maximum tolerated range in any case, calculate the average conductivity from the four replicates. Locate the average in column 1 or 2 of the table and read off the maximum tolerated range in column 3.

The tolerances take into account the experimental error between laboratories participating in comparative tests completed by the Vigour Committee 1998–2001.

Table .17

Average conductivity (µS cm^{-1} g^{-1})		**Maximum range**
from	to	
1	2	3
10	10.9	2.0
11	11.9	2.1
12	12.9	2.3
13	13.9	2.4
14	14.9	2.5
15	15.9	2.7
16	16.9	2.8

17	17.9	3.0
18	18.9	3.1
19	19.9	3.2
20	20.9	3.4
21	21.9	3.5
22	22.9	3.7
23	23.9	3.8
24	24.9	4.0
25	25.9	4.1
26	26.9	4.2
27	27.9	4.4
28	28.9	4.5
29	29.9	4.7
30	30.9	4.8
31	31.9	4.9
32	32.9	5.1
33	33.9	5.2
34	34.9	5.4
35	35.9	5.5
36	36.9	5.6
37	37.9	5.8
38	38.9	5.9
39	39.9	6.1
40	40.9	6.2
41	41.9	6.4
42	42.9	6.5
43	43.9	6.6
44	44.9	6.8

45	45.9	6.9
46	46.9	7.1
47	47.9	7.2
48	48.9	7.3
49	49.9	7.5
50	50.9	7.6
51	51.9	7.8
52	52.9	7.9
53	53.9	8.0

This table indicates the maximum difference in conductivity readings that is tolerable between tests completed on the same sample in the same laboratory. To determine if the two tests are compatible, calculate the average of the two test results and locate this in columns 1 or 2 of the table. The tests are compatible if the difference between the conductivity readings in the two tests does not exceed the tolerance given in column 3.

Table 18 Tolerances for conductivity tests on different submitted samples when tests are made in different laboratories (two-way test at 5 % significance level)

Average conductivity (μS cm^{-1} g^{-1})		**Maximum range**
from	**to**	
1	2	3
10	10.9	3.6
11	11.9	3.8
12	12.9	4.0
13	13.9	4.2
14	14.9	4.4
15	15.9	4.6
16	16.9	4.8
17	17.9	5.0
18	18.9	5.2

19	19.9	5.4
20	20.9	5.6
21	21.9	5.8
22	22.9	6.0
23	23.9	6.2
24	24.9	6.4
25	25.9	6.6
26	26.9	6.8
27	27.9	7.0
28	28.9	7.2
29	29.9	7.4
30	30.9	7.7
31	31.9	7.9
32	32.9	8.1
33	33.9	8.3
34	34.9	8.5
35	35.9	8.7
36	36.9	8.9
37	37.9	9.1
38	38.9	9.3
39	39.9	9.5
40	40.9	9.7
41	41.9	9.9
42	42.9	10.1
43	43.9	10.3
44	44.9	10.5
45	45.9	10.7
46	46.9	10.9

47	47.9	11.1
48	48.9	11.3
49	49.9	11.5
50	50.9	11.8
51	51.9	12.0
52	52.9	12.2
53	53.9	12.4

This table gives the maximum difference in conductivity reading that is tolerable when tests are completed in different laboratories. To determine if the tests are compatible, calculate the average of the test results and locate this in columns 1 or 2 of the table. The tests are compatible if the difference between the conductivity readings does not exceed the tolerance given in column 3.

The tolerances take into account the experimental error between laboratories participating in comparative tests completed by the Vigour Committee 1998–2001.

Accelerated ageing germination test

Table19 Maximum tolerated range between two replicates of 100 seeds in one accelerated ageing germination test (two way test at 2.5 % significance level). The tolerances are extracted from Table G1, column L, in Miles (1963)

Average germination percentage		**Maximum range**
from	**to**	
1	2	3
99	2	–*
98	3	–*
96–97	4–5	6
95	6	7
93–94	7–8	8
90–92	9–11	9
88–89	12–13	10
84–87	14–17	11
80–83	18–21	12
76–79	22–25	13

69–75	26–32	14
55–68	33–46	15
51–54	47–50	16

* cannot be tested

This table indicates the maximum range (i.e. difference between highest and lowest) in germination percentage tolerable between replicates in a germination test following accelerated ageing. To find the maximum tolerated range in any case, calculate the average percentage, to the nearest whole number, of the two replicates (form 100 seed replicates by combining two subreplicates of 50 seeds). Locate the average in column 1 or 2 of the table and read off the maximum tolerated range opposite in column 3.

Table20 Tolerance for two accelerated ageing tests on the same submitted sample when tests are made in the same laboratory each on 200 seeds (two-way test at 5 % significance level)

Average germination percentage		**Maximum range**
from	to	
1	2	3
99	2	–*
98	3	–*
97	4	6
96	5	7
95	6	8
93–94	7–8	9
91–92	9–10	10
89–90	11–12	11
86–88	13–15	12
83–85	16–18	13
79–82	19–22	14
74–78	23–27	15
68–73	28–33	16
55–67	34–46	17
51–54	47–50	18

* cannot be tested

This table indicates the tolerances for the germination percentage after accelerated ageing when tests are made on the same sample in the same laboratory. To determine if the two tests are compatible, calculate the average percentage of the two test results to the nearest whole number and locate this in column 1 or 2 of the table. The tests are compatible if the difference between the percentages obtained in the two tests does not exceed the tolerance given in column 3.

Table21 Tolerance for accelerated ageing tests on different submitted samples when tests are made in different laboratories each on 200 seeds (two-way test at 5 % significance level)

Average germination percentage		**Maximum range**
from	to	
1	2	3
99	2	–*
98	3	–*
97	4	–*
95–96	5–6	8
94	7	9
92–93	8–9	10
90–91	10–11	11
88–89	12–13	12
85–87	14–16	13
82–84	17–19	14
79–81	20–22	15
74–78	23–27	16
68–73	28–33	17
57–67	34–44	18
51–56	45–50	19

* cannot be tested

This table gives tolerances for the germination percentage after accelerated ageing when tests are made in different laboratories. To determine if tests are compatible, calculate the average percentage of the test results to the nearest whole number and locate this in

columns 1 or 2 of the table. The tests are compatible if the difference between the percentages does not exceed the tolerance given in column 3.

Radicle emergence test

Table22 Tolerances between highest and lowest radicle emergence of two replicates of 100 seeds in one radicle emergence test (two-way test at the 2.5 % significance level).

Average radicle emergence of test		**Tolerance**
51–100 %	0–50 %	
99	2	4
98	3	5
96–97	4–5	6
95	6	7
93–94	7–8	8
90–92	9–11	9
88–89	12–13	10
84–87	14–17	11
81–83	18–20	12
76–80	21–25	13
69–75	26–32	14
55–68	33–46	15
51–54	47–50	16

This table gives tolerances between highest and lowest radicle emergence of two replicates of 100 seeds in one radicle emergence test.

Table23 Tolerances between results of two radicle emergence tests of 200 seeds on the same or a different submitted sample when tests are made in the same laboratory (two-way test at the 2.5 % significance level).

Average radicle emergence of 2 tests		**Tolerance**
51–100 %	0–50 %	
99	2	2
98	3	3
96–97	4–5	4
94–95	6–7	5

91–93	8–10	6
87–90	11–14	7
82–86	15–19	8
75–81	20–26	9
64–74	27–37	10
51–63	38–50	11

This table gives tolerances between results of two radicle emergence tests of 200 seeds on the same or a different submitted sample when tests are made in the same laboratory.

References

1.Chalam, G.V. Amir Singh & J.E. Duglas (1967) Seed Testing Manual. Indian Council of Agricultural Research &United States Agency for International Development: (130-156)
2.Gupta D.G. (1980) Tolerances and their use in India Seed Industry Seed Tech News, Indian Society .Seed Technology 10 (4) : 10-23.
3.ISTA (1965) International Rules for seed Testing Seed Science & Technology 13 (2) : 307-520.

4.IST A (1999) International Rules for Seed Testing Seed Science & Technology; 27: Supplement 285- 297

5. ISTA (2022) International Rules for seed Testing Seed Science & Technology 6.6 :6.5

6. ISTA (2022) International Rules for seed Testing Seed Science & Technology 15.8.5-15-16

7.Miles, S.R. (1963) Hand Book of Tolerances and measures of precision for seed Testing. Proc. IntI. Seed Test. Assoc. 28: 525-686

8.Oren L. Justice and Earl E. Houseman (1961) seeds, the year book of Agriculture the united state department of Agriculture (457-462)

9.Singh D.P. and Agrawal P.K. (1993) Hand Book of Seed Testing. Department of Agriculture and Cooperation Ministry of Agriculture Govt. of India (144-185)

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New approaches to seed Vigour testing

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Increase in agricultural production is the key to overall economic growth. One of the prerequisites of efficient crop production is the use of quality seed which could guarantee assured plant stand and crop establishment. Abiotic (low/high temperature, water logging, nutrient or drought stress) and biotic stresses (disease incidence) during seed production affect seed germination, field emergence and final plant stand. Seed lots performing well under laboratory conditions may not perform satisfactorily under field conditions. Thus seed companies, seed growers and seed producers use vigour information as an additional quality assurance tool to assess their seed lots for field and storage potential.

Standard Germination Test (SGT) has been standardized for most crop species so laboratories obtain uniform reliable and repeatable results when testing seeds of same lot. However, conditions of Standard Germination Test are almost similar to the conditions to which seeds are normally subjected in the field. In many instances, seed lots of apparently equal quality as indicated by Percent Germination will produce largely different responses in field emergence (Table 1). Therefore, determining "the degree of aliveness" of the seed is just as important as determining whether "the seed is alive". Clearly, germination test per se is not enough to assess seed quality.

Table 1: Examples of the large difference between field and laboratory germination test

Crop	Lot	Lab germination (%)	Field Germination (%)
Pea	A	70	32
	B	65	69
	C	45	42
Onion	A	63	60
	B	65	27

In one of the first attempt to conceptualize seed vigour, Isley (1957) defined it as "*the sum total of all seed attributes which favour stand establishment under unfavorable field conditions*". Similarly International Seed Testing Association (ISTA) defines seed vigour as "*the sum of those properties that determine the activity and performance of seed lots of acceptable germination in a wide range of environments (Anon, 2023)*".

Seed vigour is not a single measurable property, but is a concept describing several characteristics associated with the following aspects of seed lot performance:

- Rate and uniformity of seed germination and seedling growth;
- Emergence ability of seeds under unfavorable environmental conditions;
- Performance after storage, particularly the retention of the ability to germinate.

A vigorous seed lot is one that is potentially able to perform well even under environmental conditions which are not optimal for the species. Seeds which perform well are termed as 'High Vigour' seeds (Perry, 1972).

Additional definitions

- Seedling emergence the emergence through the soil or other planting medium of a young plant developing from the embryo of the seed
- Seedling performance the ability of a seedling to emerge from the soil or other medium and develop into a normal plant
- Total germinated seeds the sum of the proportion of seedlings classified as normal and abnormal at the end of a controlled deterioration (CD) germination test conducted under the conditions and within the period specified in Table 5A.

Factors affecting seed vigour

Seed quality and vigour is greatly influenced by environmental factors, genetic factors, seed size, physiological maturity, production and storage. The major cause of the loss in vigour is attributed to deterioration and aging which commences at the time the seed become physiologically mature and it is imperative that the seed is handled carefully to prevent an accelerated reduction in performance. Physical damage to cell membranes, particularly in large seeds such as legumes, is usually the primary cause of seed deterioration. Enzyme activity in immature and dormant seeds, respiration during harvest and storage, impaired protein and the use of protein and RNA synthesis during periods of low temperature stress, genetic damage and the accumulation of toxic metabolites are all associated detrimental factors.

Vigour is not a single measurable property like germination, viability, but rather a quantitative attribute, controlled by several factors that affect the germinating seed or subsequent seedling development. It is important to note here that a vigour test cannot replace a germination test but rather supplement it with more information about seed quality. Thus vigour tests in conjunction with a germination test can give seed sellers and buyers more confidence when choosing seed lots.

Objectives of vigour tests

The relatively simple and rapid laboratory tests, in some way nor the other, indicates seed vigour. Are such tests available and how can their reliability and accuracy be determined? The only way out is to establish relationship between laboratory seed vigour test results and field performance and/or seed storage potential.

Any seed vigour test should largely comply with the following objectives (ISTA HANDBOOK OF VIGOUR TEST METHODS, 3RD EDITION 1995):

- To provide a more sensitive index of seed quality than the germination test.

- To provide a consistent ranking of seed lots in terms of their potential performance.
- To be objective, rapid, simple and economically practical.
- To be reproducible and repeatable.

Seed vigour testing methods

As early as 1876, Friedrich Nobbe described vigorous "Triebkraft" a German word meaning "Driving Force". Scientists have long searched for a way to describe the differences amongst seed lots with comparable germination. Numerous tests, theories and methodologies have been used to test for, and describe seed vigour. Over the years, many seed vigour tests have been developed and evaluated. A simple classification is given in Table 2. Sometimes a combination of tests is used on a seed lot to read its planting potential more accurately.

Table 2: Classification of seed vigour tests

Seed appearance	Performance Based	Stress Based	Biochemical based
Seed Size	First Count	Cool germination test	GADA test
Seed lustre	Speed of germination	Cold germination test	TZ Test
Seed density	Seedling growth rate and dry weight	Brick gravel test	Respiratory quotient [R.Q.]
		Paper piercing test	Membrane integrity test
		Accelerated ageing test	
		Controlled deterioration test	

I. Performance based Tests

A. First Count

It is to be used to compare different seed lots over several months. The number of normal seedlings removed when the preliminary count (First Count) of the germination test is made and it is the indication of the seed lot's quality. Here, the higher the percentage of normal seedling at first counts, the better the seed quality.

B. Speed of Germination

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

For e.g. the quality indexes of Lot A and Lot B is obtained in the following manner:

$$Lot\ A = \frac{\text{No. of Seedling removed}}{\text{Day After Planting}}$$

$= 0/1 + 0/2 + 0/3 + 8/4 + 10/5 + 24/6 + 28/7 + 24/8$

$= 0+0+0+2+2+4+4+3 = 15$

$$Lot\ B = \frac{\text{No. of Seedling removed}}{\text{Day After Planting}}$$

$= 0/1 + 0/2 + 12/3 + 24/4 + 45/5 + 7/6$

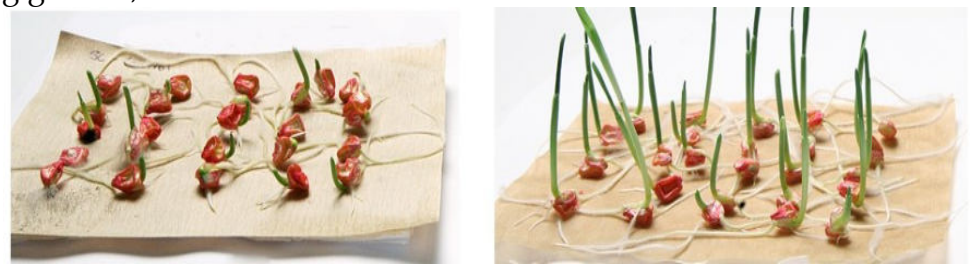
$= 0+0+4+6+9+1 = 20$

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

High and low vigour seed lots of Wheat

C. Seedling Growth Rate and Seedling Dry Weight

Measurement of seedling growth rate greenhouse or field or laboratory conditions on a specified number of days from planting indicates its vigour. The blotters along with planted seeds are placed in a seed germinator cabinet at 20^0C in dark, for a fixed number of days. The length of root and shoot of the normal seedlings are recorded, and the seed lot producing the most growth is considered the best quality. The seedlings are dried at 110^0C for 17±1 hrs to record their dry weight. Better quality is considered to produce higher seedling dry weight. The advantage of such test is that seed testing laboratories do not require additional equipment and that little additional training of staff is required. The disadvantage is that variables such as humidity and temperature, which exert a significant influence on seedling growth, are difficult to standardize.



Low and high vigour seed lot of Maize

II. Stress based tests

Under favorable field conditions, a good correlation usually exists between standard germination test and field emergence. Under unfavorable conditions, high vigour seeds have a greater potential for emergence. Consequently a number of vigour tests, which assess performance under stress conditions, have been developed.

A. Cool germination test

The test is limited to measuring the effect of cool temperature on the germination of cotton seed and the growth rate of cotton seedlings. Germination test is conducted at constant temperature of 18^0C and sufficient humidity. Only one count is recorded, on sixth day for acid delinted cotton seed and on the seventh day for machine delinted cotton seed.

B. Cold germination test

The whole idea behind a cold test is to evaluate the emergence of a seed lot in cold wet soils, which can cause poor field performance. It is the most widely used vigour test for maize and other crops (soybean, sorghum) and is also widely accepted by the seed industry in other parts of the world. The cold test is one of the oldest vigour tests (Isley, 1950). The cold test determines the ability of seeds to germinate and produce normal seedlings under two stress conditions viz. a sub-optimal temperature (Cold moist conditions) and pathogen (soil borne pathogens). At low temperatures, low vigour seeds tend to leak out more sugars, amino acids, etc. and encourage growth of pathogen. The ability to perform well in cold wet soils id influenced by genotype, mechanical damage, seed treatment and physiological condition of the seed, the cold test determines the combined influence of these, and possibly other, factors.

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

Cold test in maize

C. Brick gravel test

"The Hiltner Brick Grit Test" or "The Hiltner Test" was originally developed by Hiltner and Ihssen (1911) for seed-borne infection by *Fusarium* spp. after it was observed that coleoptiles from infected, germinated seeds were short and not able to penetrate a 3 cm thick layer of brick grit without physical damage. It can also been used to test cereals injured by sprouting, seed treatment, threshing damage and frost damage.

Seeds infected by pathogenic fungi, injured seeds, or those low in vigour are often weak and unable to withstand adverse conditions during germination and field emergence. The brick grit layer used in the Hiltner test imposes a physical stress on the seeds. Seedlings which emerge normally through brick grit are considered to have been able to withstand the physical stresses involved, and the test thus provides a method to screen seed lots for vigour.

D. Paper piercing test

This method utilizes regular testing sand plus a special type of paper disc which seedlings must penetrate to be considered strong. The paper must have the following characteristics:

- Basic weight : 90g/m^2
- Thickness : 0.4mm
- Dry Bursting Strength : 0.3 kg/cm^2
- Wet Bursting Strength : 150 mm
- Breaking Strength : 1000-5000 mm
- Filtering Speed : 500 ml/min
- Ash content : 0.1 %

The test is used for cereal crops requires placing seed on top of approximately 1.2 cm of moist sand, covering seeds with a special dry filter paper, and covering the paper with about 3 cm of moist sand. Test is carried out at 20^0C for eight days.

E. Accelerated Ageing Test

Accelerated Ageing Test (AAT) was initially developed as a test to estimate the longevity of seed in commercial storage (Delouche and Baskin, 1973). Studies have shown that results are correlated with emergence of cotton, peas, beans and soybean. It is recommended by ISTA Seed Vigour Testing Committee as a vigour test for soybean. Here, the seeds are exposed to an elevated temperature (40-45^0C) and high relative humidity (greater than 90%) for 48 hours or longer, depending on the species. Under these conditions seed deterioration is accelerated, with least damage to high vigour seeds. After the ageing period, which may be different for different crop species, the seed is planted in germination test conditions. This is ISTA recommended vigour test for soybean.

F. Controlled Deterioration Test

This test is similar to Accelerated Ageing test, except that seeds are pre-conditioned to a specified moisture content (say, 20 %) and then sealed in aluminium foil - Polyethylene seed pouches which are submerged in water bath at a high temperature (40-45^{0}C) for 24-48 hrs. the percentage germination is determined subsequently, and the results obtained is correlated with field emergence and storage potential of various vegetable crops. This is ISTA recommended vigour test for *Brassica* sp.

III. Biochemical Tests

Radicle emergence and subsequent seedling growth are the end result of metabolic reactions. It follows that the determination of metabolic activity should provide a measure of seed vigour. Biochemical tests are more rapid than other vigour tests but require specialized equipment and training.

A. GADA TEST

The Glutamic Acid Decarboxylase Activity(GADA) Test measures the activity of one specific enzyme and the level of enzyme activity is determined by the amount of carbon dioxide (CO_2) given off and is positively related to seed quality; i.e., the more the CO_2 evolved, the better the seed quality (Grabe, 1965).

B. TZ Test

The Tetrazolium test is one usually used to test seed lot viability. This test can also be very useful in determining seed lot vigour. The test is basically carried out the same as the viability test but viable seeds are evaluated more critically into categories of High, Medium and Low vigour as follows:

High Vigou : Staining is uniform and even, tissue is firm and bright.

Medium Vigour : Embryo completely stained or embryonic axis stained in dicots.

Extremities may be unstained. Some over stained/less firm areas exist.

Low Vigour : Large areas of non-essential structures unstained. Only one root stained (monocots) or extreme tip of radicle unstained (dicots). Tissue milky, flaccid and over stained.

A major benefit of the test is a very rapid estimate of the vigour of viable seeds. To achieve meaningful results, a very experienced analyst must evaluate the test and precision of methods must be strictly adhered to. This test has been used extensively on cereal crops and results for field pea have shown good relationships with field performance. It is also used in soybean, cotton, corn, and large seeded legumes.

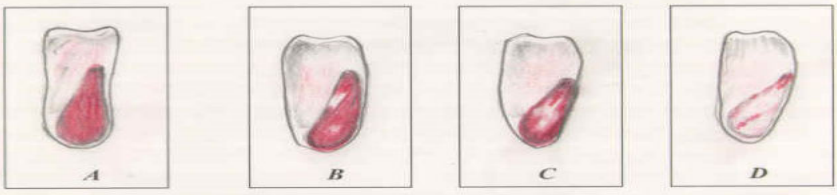


Figure 2. Examples of high (a), medium (B), low (C), and ungerminable (D) corn seeds using the TZ as a vigor test.

C. Respiration [R.Q.]

During the process of respiration, oxygen is taken up by seeds and CO_2 is released. The ratio of the volume of evolved per unit time to the volume of oxygen consumed per unit time is called Respiratory Quotient (RQ). The RQ were found to be more often related to the vigour than oxygen uptake alone. In corn and paddy, differences in respiration rate have been used to distinguish between high, medium and low vigour seeds. The rate of gas exchange is measured in the Warburg respirometer / apparatus.

D. Membrane Integrity Test

Also called as Conductivity Test, provides a measurement of electrolyte leakage from tissues and was first recognized by Hibbard and Miller (1928). Seed deterioration is associated with loss of membrane integrity. When deteriorated seeds are soaked in water they lose more electrolytes (amino acids, sugars, organic acids etc.) which increase the conductivity of water. A high conductivity of this solution denotes low vigour. In the standard conductivity test, results are expressed as mean conductivity per gram of seed weight obtained after soaking 25-50 seeds for 24 hours. This test has proved highly successful on garden pea but good results have also been resulted have also been obtained with large seeded legumes and grains. This test has been recommended by ISTA Seed Vigour Testing Committee for garden peas.

Conductivity Test results can be used to rank lots by vigour level. This information has been used for several years for garden peas and is interpreted as follows (Matthew and Powell, 1981).

EC value	Remarks
<25 uScm^{-1}g^{-1}	indicate seed suitable for early sowing or for sowing in adverse conditions
25-29 uScm^{-1}g^{-1}	Seed may be suitable for early sowing, but there is some risk of poor performance under adverse conditions
30-43 uScm^{-1}g^{-1}	Seed not suitable for early sowing especially under adverse conditions
>43 uScm^{-1}g^{-1}	Seed not suitable for sowing

Advantages of seed vigour tests

1. Seed vigour tests rank seed lots for physiological quality
2. The results provide information which can be used to plan strategy with respect to carryover potential of seed lots during further storage and/or marketing.
3. Seed vigour tests are a great tool for in-house quality control.

Limitations of seed vigour tests

1. A precise referee testing method among seed laboratories is needed to arrive at a common vigour test methodology.
2. Seed vigour tests do not predict percentage field emergence.
3. Values obtained from seed vigour tests are relatively values, not absolute values of vigour.
4. Comparison of the results of different tests is difficult because results are expressed in different units.
5. Exact points between acceptable and unacceptable levels of vigour have only been established for a few recommended tests (e.g. conductivity test for garden peas) and must be established for other frequently used vigour tests.

References

- Anonymous 2023 International Rules for Seed Testing *Seed Science and Technology*: 15-i to 15-xiv
- GRABE DF 1965 Prediction of relative storability of corn seed lots.*Proc Assoc Off Seed Anal***55**: 95-96
- ISLEY D 1957 Vigour tests *Proc Assoc Off Seed Anal***47**: 177-182
- PERRY DA 1972 Seed vigor and field establishment *Hort Abstr***42**: 334-342
- POWELL AA and Mathews S 1977 Deteriorative changes in pea seeds (*Pisumsativum*) stored in humid or dry conditions *J Expt Bot***28**: 225-234
- POWELL AA and MATTHEWS S 1981 Evaluation of controlled deterioration, a new vigour test for crop seeds*Seed Science and Technology* 9: 633-640
- SRIVASTAVA JP and SIMARSKI LT (eds) 1986 Seed Production Technology *International Centre for Agricultural Research in the Dry Areas (ICARDA)* Aleppo, Syria
- VAN DE VENTER A 2001 Seed Vigour Testing*Journal of New Seeds***2(4)**: 51-58
- WOODSTOCK LW 1969 Seedling growth as a measure of seed vigor *Proc Intl Seed Test Ass* **34**: 273-280.

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Physical purity and ODV testing in seed samples

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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 Agropyron repens* 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., Agropyron cristatum, Agropyron desertorum 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 Dactylis glomerata 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 *Dactylis glomerata*, one-fifth the weight of multiple florets (see *Uniform Blowing Method*).

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

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

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 crops except rice
- For rice 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

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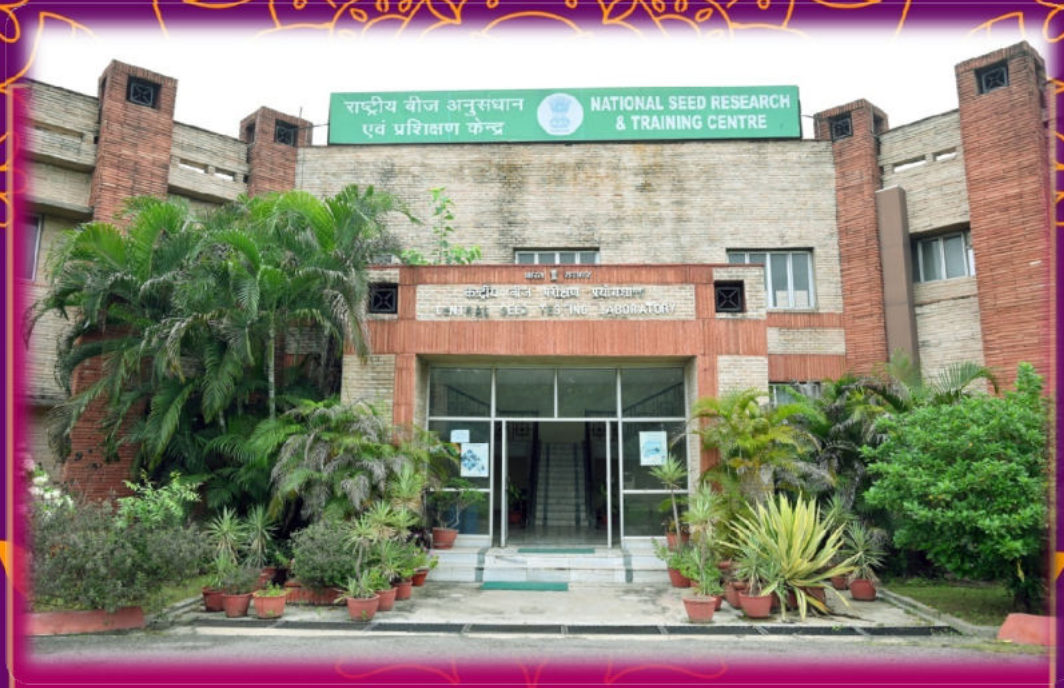

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